Potential Alternate Treatment Systems

Lead: Dr. Richard Warner, U of Kentucky

P.O. # 750000223 and 7500011726

Final Report: Se Removal from Coal Mine Valley Fill Effluents: ARIES Subtask 2.2.3

Revised 21 November 2013

www.boojumresearch.com

Boojum Research Limited

Final Report

Selenium Removal from Coal Mine Valley Fill Effluents using *Chara*

ARIES Subtask 2.2.3

July 31, 2013

Revised 21 November 2013

Boojum Research Ltd.

302-21 St. Clair Ave. East

Toronto, Ontario, Canada M4T 1L9

Executive Summary

In the scoping study conducted in 2012, the applicability of using ecological engineering approaches to lower or remove selenium from valley fill ponds and effluents, based on selenium biochemistry literature was examined. Ecological engineering methods utilize natural, biological processes to alter biogeochemistry and remove selenium from wastewater. The most promising algae, based on literature and the scoping study, was to cultivate the alga *Chara* in the valley fill ponds to adsorb/absorb selenium. The sequestered selenium would follow the *Chara* as it died into the sediments, where it would remain, under anaerobic conditions until the pond was dredged.

Of the four Industrial Affiliate ponds, only Arch Coal's Black Castle Pond 3 (upper of the two ponds in series and monitoring station WV1013441) had an abundant *Chara* population. Black Castle Pond 2 (the lower of the two ponds in series monitoring station WV 1912441)) also called Trout Pond had a much smaller *Chara* population. The effluent from the two ponds in series (first 3 then 2) was monitored by Black Castle as discharge points. .

Arch Coal's Black Castle Pond 1 (monitoring station WV1020358**), c**alled locally Morgan's pond was dredged in 2012. This pond had little vegetation, no *Chara*, and less organic sediments. As expected, the selenium concentrations leaving the pond were the same as entering the pond. Without any biological 'filters' to remove the selenium, it passes straight through.

The Cliffs Resources' Cliffs-Dingus Pond (monitoring station WV1016750) was just the opposite of Black Castle Pond1. It was 'choked' with vegetation. Most of the vegetation was aquatic and emergent vegetation, not *Chara*. These plants also removed selenium, but have the distinct disadvantage that as leaves and stems die, they are not incorporated in the sediment, but can decay and re-dissolve into the water column, thereby releasing selenium. Root systems can also translocate already sediment-bound selenium into the leaves and stems and ultimately back into the water column. No *Chara* was found in Cliffs-Dingus Pond 1.

Chara was transplanted by Boojum Research, October 2012, into Black Castle Ponds 1 and 2 and Cliffs-Dingus Pond 1. However, by June 2013 none of *Chara* had survived or could not be found due to high turbidity. The electrical conductivity of the valley fill ponds was much higher than the ponds from which the biomass was collected. In 2013 biomass from valley fill ponds was used and transplanted in nets with floats. We summarized the water and biomass elemental composition of other mine waste water ponds colonized by Chara. The composition to the algae and the water of the valley fill ponds showed no obvious differences. We therefore conclude that Chara would grow when introduced as a primary colonizing species in the valley fill ponds. A publication was submitted for peer review and is under revision, presented in Appendix 2.

Estimates of removal capacity of *Chara* in Black Castle Pond 3 were derived based on very limited flow and selenium data. Confirmation is needed to refine estimates. Selenium concentrations are remarkably lower in the outflow than the inflow, reporting a reduction of 7 µg L^{-1}. The selenium concentration in *Chara* ranged from 2.2 to 8.0 mg.Kg^{-1}. Chara removed between 0.07 grams of Se per square meter in Black Castle Pond 2 (sparsely colonized pond) to 0.9 g m^{-2} in Black Castle Pond 3 (more densely populated) ponds .Sediment core analysis was conducted for Cliffs Pond 1. The highest selenium concentrations were found in the surface horizon (0-2 cm) 17 µg.g^{-1}, decreasing to 7 µg.g^{-1} at a depth of 2-4 cm with a further decrease to 1.37 µg.g^{-1} in the next 2 cm segment and finally below 6 cm, concentration of 0.6 µg.g^{-1} was found. The decreases with depth demonstrate the accumulation of selenium on the pond sediment.

Conclusions:

1. Overall the three fundamental premises required to utilize a *chara*-based selenium removal system were confirmed: a) *Chara* can form extensive coverage in valley fill ponds (Black Castle Pond 3), b*) Chara* is effective in adsorbing/absorbing a large quantity of selenium and c) organic deposited sediments do contain elevated selenium and thus can and will retain *Chara* degraded biomass.
2. Black Castle Pond 3 has a well established *Chara* population which is effective in selenium removal.
3. Valley fill ponds with an established and growing charophyte population have a high potential to provide a stable, long-term, cost-effective selenium removal system.

Recommendations:

Based on the data collected and observations made, we would recommend that a valley fill pond be used for a pilot study after it is dredged free of the accumulated sediment and immediately seeded with *Chara* oospores concentrated from local sediment and or with *Chara* biomass. A large-scale transplant will be successful, contrary to field trials where a small amount of *Chara* biomass had to competing with other vegetation.

Along with the introduction of biomass reliable hydrological data needs to be collected for the pilot test pond to determine accurate selenium loadings to the algal biomass. These include quantification of inflow and outflow flow of effluent and accompanying recording of atmospheric precipitation. Particle or TSS values need to be quantified with sedimentation traps, as debris in the pond will also collect Se, as was noted from the washings obtained. The predictions by Ziemkiewicz and Lovett (2011) indicate that the [Se] in valley fill effluents are decreasing below regulatory limits within 25 years. This supports further a pilot scale field test with Chara introduction, as it is an economic and sustainable solution.

Margarete Kalin, Dr. William N. Wheeler and Dr. Robin Scribailo 25th of September 2013 submitted, revised R. Warner – December 8th 2013

Table of Contents

List of Figures

List of Tables

Introduction

In the scoping study carried out in 2012, we examined the applicability of using ecological engineering approaches to lower or remove selenium from valley fill ponds and effluents, based on selenium biochemistry literature. Ecological engineering methods utilize natural, biological processes to alter biogeochemistry and remove selenium from wastewater. These biological processes include the transformation of metalloids by fungi and bacteria. Such transformations have been utilized successfully to decontaminate soil and water of selenium from the San Joaquin Valley and the Kesterson Reservoir in California. Other processes include the volatilization of selenium by vascular plants, algae and microbes. We summarized in the scoping study three major processes that might be utilized for selenium removal in valley fill ponds:

(1) **Volatilization** - Many emergent macrophytes, algae and microbes can reduce selenium to an organic form, and respire it – sending it directly to the atmosphere.
(2) **Transformation via anaerobic microbes -** Selenium is taken up and/or adsorbed by aquatic macrophytes, algae, moss and fungi and can be reduced to elemental selenium after deposition in anaerobic sediments.
(3) **Transformation via aerobic microbes** - which transform selenium directly to elemental selenium?

We visited several valley fill ponds in the scoping study and found many of them teeming with life and, in some, the desired macrophytic alga, *Chara* was also present. This alga displays a growth habit which favours biomineralisation of selenium due to a chemically-active cell wall. Our database of biota in mining waste waters summarized during the scoping study indicated that in most of the environmental compartments we studied (*Chara* biomass, suspended solids, sediment and biofilms) contained selenium, even when water selenium concentrations were below the analytical detection limit.

Some of the valley fill ponds encountered during the scoping study contained a high concentration of suspended solids. These suspended solids impaired visibility and reduced the depth at which aquatic plants and algae could grow. This restricted most of the biomass to the shoreline areas. The plants we collected for further study were covered with these suspended solids, suggesting that there might be a difference between unwashed and washed plant selenium concentrations.

The plant material and debris we collected were put through a standard washing procedure. It is reasonable to assume that material which can be washed off a plant would be similarly released during a storm event, and thus could be caught by a sedimentation curtain. Both the washed debris and the plant organic matter were analysed for selenium, and both contained the element. This suggests that selenium is both bound to organics and to particulates, suggesting further that any treatment system has to deal with both.

Practically speaking, while all three selenium transformation processes probably exist in the ponds, we do not know which are prevailing and dominant. Are the ponds internally cycling selenium? Are the sediments sinks for selenium, as projected from the literature? Ecological engineering technology recognizes and utilizes the ecological capacity to clean water through plant, microbial and fungal involvements. However in light of the complex biogeochemistry of selenium, the identification of the dominant processes and which geochemical species contributes to the measured selenium concentration cannot be addressed in this study. The Se distribution is of an empirical nature as opposed to geochemical in the pond ecosystem.

In this second study, we describe the ponds from an ecological perspective and determined selenium concentrations in relevant environmental compartments. Finally, we used flow and selenium loadings (flow times selenium concentration) and estimated removal rates in relation to the growth rates of the major biota present.

In order to use the ponds effectively as a treatment system, we needed to determine the "pathway or distribution" of the selenium in the pond ecosystem. We therefore seek answers to the questions posed below by interpreting the data which we have collected from sampling the ponds.

- Is the incoming water higher, lower or unchanged in [Se] than the water leaving the pond?
- If the outflow [Se] is **higher** than the inflow [Se] :
    - Then could the sediments be the source?
    - Then could the decaying plants debris be the source?
    - Or could the [Se] come from a different additional water source?
- If the outflow [Se] is **lower** than the inflow [Se]:
    - Is the Se carried by the debris to the sediment?
    - Is the Se in the perennial roots of the rooted plants?
    - Is the Se in the annual parts of the rooted plants?
    - Is the Se on the epiphyton, which might be liberated to the water by waves?
- If the outflow selenium concentration is unchanged when the water is leaving the pond:
    - Are there any obvious differences to the other two described conditions?

For an effective ecological treatment, the source and sink of the selenium have to be determined. Normally, a minimum of 4 sampling trips are scheduled in a year to ensure that the entire range of biological and physical conditions of the pond ecosystem are sampled. However, due to the limited time available, an inability to sample many of the same ponds sequentially, and the small number of ponds in the study, it was only possible to identify more general ecological processes likely to be important in the development of an effective biological treatment system. We have collected water, plants, debris and sediments. Each of these ecological components will give different information. The following components were analyzed:

Water: Water was collected in all four ponds and analyzed for total recoverable concentrations and concentrations determined after 0.2 µm filtration.

Plants: Plants and floating debris were collected in all four ponds and epiphytes (growth on the surfaces, sediment curtains or plant parts) were washed off and analyzed.

Sediments: Sediment cores were collected in Cliffs Pond and in Black Castle Pond 1 pond, covering a depth from the surface to 10 cm depth and from surface to 24 cm depth, respectively separating both cores into 2 cm units.

The very first step is to determine if the ponds are different, with respect to inflow and outflow concentrations of selenium. These differences, or the absence thereof, can be attributed to many factors. It is essential to determine if differences in selenium outflow concentration are attributable to internal hydrological and chemical processes within the ponds or inflow characteristics. Since little hydrological data are available the results and interpretation must be treated with caution. Phase II of ARIES was supported by providing access to a total of 4 ponds for ecological assessment. One pond belonged to Cliffs Resources (Cliff -Dingus 01) and three ponds belonged to Alpha Natural Resources (Black Castle Ponds 1, 2, and 3).

1.1 Scope of Work

1. **Acquire design Parameters for 2-3 valley fill ponds**
    a. Do an enhanced site assessment, including water quality measurements
    b. Sample water, deposited sediment and vegetation
2. **Identify biomass**
    a. Algae
    b. Aquatic vegetation
3. **Transplant vegetation (primarily *Chara*) to selected valley fill ponds**
4. **Measure growth rates of transplanted vegetation.**
5. **Report/Conclusions/Recommendations for next stage**

Relevant historic information and flow and loading data were to be derived from monitoring records and from work of Dr. Warner's group. Environmental chemistry was to be provided by Dr. Unrine's laboratory at the University of Kentucky.

2.0 Methods

Black Castle Pond 1 was visited on October 1st but could not be sampled because there was no available access to a 4 wheel-drive truck. Black Castle Ponds 1, 2 and 3 were sampled the following day on October 2nd and Cliffs Pond was sampled on October 3rd, 2012 and ponds were revisited in summer 2013.
.

Aquatic Plant Diversity: The diversity of aquatic plant species was assessed by navigating the entire littoral zone of the ponds using a Bass Pro Shop Uncle Buck's® pond prowler boat and periodically sampling the vegetation with a long-handled rake. Individual vegetation samples were stored for later identification. Vascular aquatic plant identification was made primarily by reference to various volumes of the Flora of North America series (2003-2012). Percent vegetation cover in the ponds was estimated visually for major vascular plants.

Plankton Tows: Two plankton tows were performed at different points in each pond using a Forestry Supply plankton net (60 micron mesh size) attached to 100 ml sample bottles. This mesh size allows capture of larger invertebrates, protozoa, and most phytoplankton. Nets were allowed to sink approximately 3 meters and were then pulled obliquely back to the boat for a distance of 6 meters. Samples were preserved by addition of small amounts of Lugol's solution. Water samples were also examined to determine the presence of smaller phytoplankton such as nano- or pico-plankton.

Periphyton: Periphyton was collected wherever they were observed on either living or dead plant material, or sediment or silt curtain surfaces. The samples were placed in pond water in zip-lock bags for later examination. All plankton samples and algae (free-floating or periphyton) were examined, as wet mounts on a Nikon YS2-T compound stereomicroscope, and where greater resolution was required a Nikon Optiphot research stereomicroscope. Samples were occasionally stained with toluidine blue or methylene blue to help distinguish the nature of the sheath in blue-green algae. Up to five slides were examined for each sample to give a qualitative estimate of the relative abundance of the different genera observed. Algae were primarily identified using Wehr and Sheath (2003) but also with reference to Prescott (1962, 1978). Invertebrates were identified using Thorp and Covich (2001).

Biomass: Biomass was collected for charophytes. Only a small number of isolated beds of *Chara* were observed at Black Castle Pond 2 so destructive sampling did not seem appropriate. Two 0.1 m^2 quadrat samples (0.33 m x 0.33 m) were taken on Black Castle Pond 3. Samples were transported to the lab and washed through cheesecloth to remove periphyton. Both fractions were then dried in a Despatch Inc. drying oven at 60^0C for one week and reweighed to determine the dry weight of *Chara* biomass versus periphyton.

Sediment Samples: Sediment sampling was carried out using a Wildco stainless steel liner type hand-corer. Slickness of sediments made it very difficult to retrieve an intact core especially at the deepest point of the pond. Sediment grabs were also attempted using a standard Wildco Ekman grab sampler. A malfunction in the spring-loading mechanism prevented use of this sampler after one initial grab (loss of the retaining screw and spring-load mechanism on one side of the Ekman sampler).

Aquatic Plant Transplants: *Chara haitensis* (stonewort) and *Ceratophyllum demersum* (coontail) were collected from the extensive beds of these species in the Valparaiso Lakes in Valparaiso, Indiana and stored in Rubbermaid containers on September 29th and 30th. The plant samples were thoroughly washed repeatedly to remove sediments. Both species are native to West Virginia and the surrounding region. Plants were wet–weighed by suspending samples in a mesh bag attached to a Rapala 50 lb fishing scale. Plants of both species have negative buoyancy so were dropped into the water at sample points. Neither species produces true roots so plants can grow from existing biomass vegetative. *Chara vulgaris* reproduces from oospores which were very abundant on the material collected. Coontail primarily reproduces through clonal growth.

In-field Water Chemistry: Depth profiles for pH, conductivity, oxygen, and temperature were carried out at 0.5 meter intervals using an YSI-85 hand-held multi-meter. The meter was initially calibrated for pH using Watermark® pH 4.00 and 10.00 buffers and for conductivity using Watermark® Conductivity Calibration Solution (FSI#76115). The calibration of the meter for these two parameters was also periodically checked in the field using YSI 5580 Confidence Solution. Temperature was calibrated using aqueous solutions of known temperature across the range of temperatures expected in the field. The oxygen membrane was replaced just prior to field work to ensure the accuracy of readings and was checked in the lab. Depth profiles were measured at several localities in each pond and the perimeter of the pond was assessed for "pop-up" regions of inflow periodically by looking for sudden changes in conductivity or temperature.

In-lab Water and Sediment Chemistry: ORP and pH was measure from water samples in the lab using an AR15 Accumet® Research (Fisher Scientific) pH meter with ORP capability. A Hanna Instruments HI3131B refillable ORP electrode was used after being initially calibrated with Zobell's reagent. ORP was measured in the core by extruding the sample in small increments and inserting the probe in the wet sediments then cutting off the later and reinserting the probe. This technique results in a minimal disruption and mixing of the sediment layers.

Data Interpretation: Environmental regulations consider a specific concentration of an element in the discharge of an industrial facility. This is somewhat limiting, since open pond systems at the bottom of differing drainages will naturally discharge water with quite different characteristics depending on the season , the weather, as well as the physical and ecological conditions of the pond. This is especially the case for essential elements such as selenium, which are widely distributed in the ecosystem with different effects at different concentrations. Therefore evaluation of concentration has to be based on carefully-evaluated analytical procedures.

Essentially, with these data we determine (on a preliminary basis) the pathway(s) of selenium as it moves through the ponds. The regulatory limit of 5 µg L^{-1} for selenium is based on the total recoverable concentration (after J. Unrine). Thus, the total recoverable selenium concentration was used throughout the pond systems. Together with selenium, other common anions, cations and trace elemental concentrations were determined. All data are presented in Appendix 1 along with graphics to facilitate comparisons of the water and biomass constituents.

Data Generation: differences in concentration were assessed, based on data produced from Dr. Unrine's laboratory at the University of Kentucky. Assessments were based on concentration of total recoverable selenium determined from water sampled at the same location, both unfiltered and filtered through 0.2 µm filter paper. Comparison of unfiltered vs. filtered concentrations enabled us to determine if the selenium was associated with particles larger or smaller than 0.2µm. Analytical error was determined by comparing the pond water data to that of the National Institute of Standards and Technology (NIST) using SRM 1643.e where known concentrations for some elements are reported. For plants and sediment cores, selected duplicate samples were run to determine the sample variability and for the analytical error, specific to the analytical run of the material, analyses of Standard Certified Material Montana soil I and Ii and SRM1-TORT-2 and SRM2 TORT 2 were carried out. The differences in concentrations between the standards in the analytical run and the detection limits of the analysis were taken into account in considering reported concentration differences. No statistical analysis of the elemental data from the ponds was possible because only a single sampling event was carried out. Nevertheless, comparison of the data with the extensive elemental information from Boojum's database, as well as information from published literature, has allowed us to identify ecological and biogeochemical processes that are likely to be important in the development of an effective approach to valley fill pond management.

3.0 Results

3.1 Pond Descriptions and Vegetation

3.1.1 Black Castle Pond 1

Figure 1. *Aerial view of (Black Castle Pond 1).*

Table 1*. Dimensions of the Black Castle Pond 1 pond*

Watershed area	60.7 ha
Storage capacity (volume)	24,400 m^3
Area	1858 m^2
Max Depth	4.5 m

Vegetation was very sparse covering only about 20% of the bottom as the pond was dredged in 2012. The main emergent plant was the narrow-leaf cattail with a small number of plants of *Scirpus* sp. and the only two submerged plants were leafy pondweed. Although there was no immediately apparent bottom film there was considerable floating decomposing detritus. Some of this material was also accumulated in some places on the bottom or was adhering to the silt curtain. The water was very opaque with lots of suspended fine silt and the bottom could not be seen below about two feet. Given the vegetation conditions this pond it will **likely not support** any of the ecological selenium removal processes.

Figure 2. *Black Castle Pond 1 from the effluent end of the pond.*

In the summer 2013 sampling, the only additional aquatic plant species recorded was curly-leaf pondweed (*Potamogeton crispus*). This species is an annual pondweed which dies back by August. It is considered an invasive aquatic plant species which spreads rapidly. It has previously been recorded for many locations in West Virginia.

Both Black Castle Pond 2 and Black Castle Pond 3 ponds are contiguous, with water flowing down from the filled valley into Black Castle Pond 3 and thence into Black Castle Pond 2. They are not connected in any way to Black Castle Pond 1.

3.1.2 Black Castle Pond 2

Table 2. *Dimensions of the Black Castle Pond 2*

Watershed area	127.5 ha
Storage capacity (volume)	23,313 m^3
Area	1742 m^2
Max Depth	7.1 m

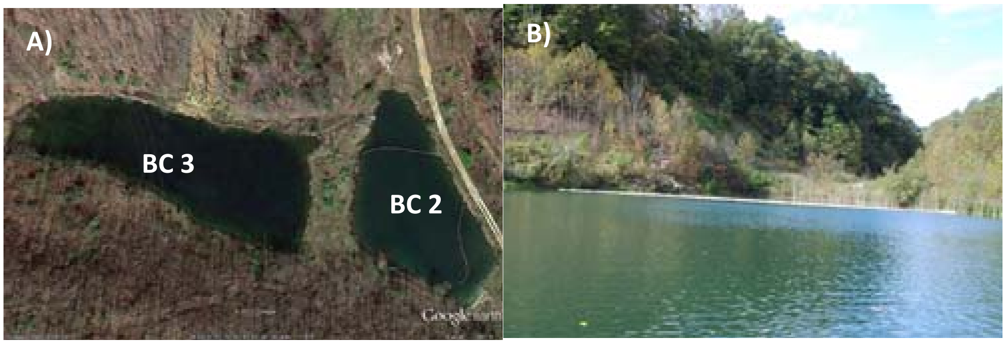


Figure 3. *A) Aerial photo of Black Castle Pond 2 and Black Castle Pond 3. B) Black Castle Pond 2 from ground opposite drainage area.*

BC 2 is deep (7.1 m) with only shallow edges which support dense populations of narrow-leaf cattail with extensive epiphytic growth on the old stocks and the silt curtain. The main submerged species were the small leaved pondweed and an unidentified pondweed (Table 5). A few small patches of common stonewort or *Chara*, the desired plant, were present.

3.1.3 Black Castle 3

Table 3. Dimensions of the Black Castle Pond 3.

Watershed area	127.5 ha
Storage capacity (volume)	24,400 m^3
Area	5806 m^2
Depth	7.8 m

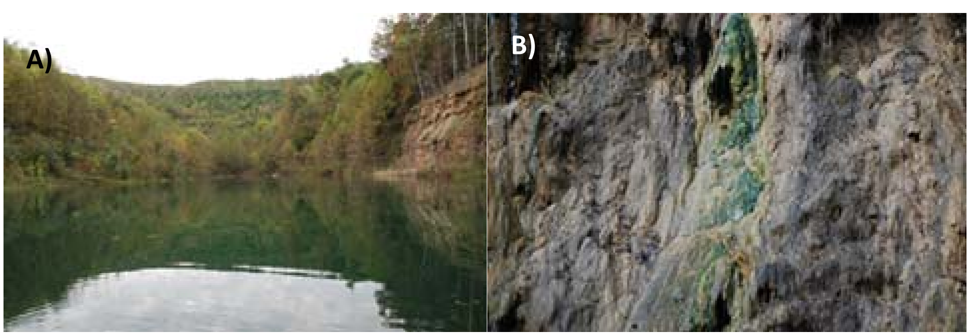


Figure 4. *A) Black Castle Pond 3, looking toward the upper side of the valley fill. B) Close up of algae growing on cliff on the right hand side of photo 'A'.*

Black Castle Pond 3 is also a deep pond with vegetation near the perimeter but more gently sloping near the inflow. The pond contains abundant Sago pondweed at both ends as well as locally dense patches of *Chara*. Submerged vegetation was 70% the former and 30% *Chara* cover sparsely on the bottom at 7.8m (Table 5). Both species were coated with periphyton and trapped considerable debris.

The cliff wall depicted on the right in Figure 4b shows biomineralized evaporates or precipitates in a water fall type configuration. Extensive algal growth occurs among minerals or precipitates. Below the waterfall are patches of phoenix moss. The flow of water coming off the cliff was not determined.

3.1.4 Cliff's Pond

Table 4. *Dimensions of the Cliffs Pond.*

Watershed area	59.9 ha
Storage capacity (volume)	7401 m^3
Area	669 m^2
Depth	3.9 m

Figure 5. *View of the Cliffs Pond, looking toward the valley fill.*

The Cliffs-Dingus Pond was by far the most highly vegetated; with a maximum depth of about 3.9 m. Eighty percent of the pond was vegetated. This vegetation was about 40% sago pondweed, 40% small pondweed and 20% water grass but no Chara was found (Table 5). The most dominant alga was *Oscillatoria* but this was the only pond to also have small amounts of the green algae *Mougeotia* and *Spirogyra*. The scarcity of green algae may also simply be a seasonal effect since this was mid-October.

Curly-leaf pondweed (*Potamogeton crispus*) was also observed. It is an annual pondweed which dies back by August. It is considered an invasive aquatic plant species which spreads rapidly, and h has been recorded for many locations in West Virginia. In addition a small area of the floating pondweed longleaf pondweed (*Potamogeton nodosus*) was also observed. In its current configuration this pond is most likely ineffective in removing selenium, since it has free floating algae, annual decaying vegetation, all biological material which would contain selenium, but through decay would release it again to the water, unless it gets carried to the sediment, where it might be bio-mineralized if the correct conditions prevail.

3.2 Vegetation Descriptions

Luziola fluitans (southern watergrass) is a perennial grass that forms dense colonies in many waters throughout the Southeastern US. It occurs in shallow water or on normally-flooded shorelines. Its leaves can be underwater (up to 1 m), floating, or emergent up to 20 cm in height, with stems up to 1 m in length. Under flood conditions, stems become erect and the leaf blades densely cover the surface of the water, giving the appearance of a firm substrate. This grass tends to be more common in disturbed areas, especially on grazed shorelines where herbivory limits the height of competing vegetation. This species is a true aquatic grass that looks like lawn grass. It is rooted on the bottom extending up to float and trail across the shallow, slow-moving waters of ponds, swamps, streams, and wet, disturbed sites nearly throughout the state. Water grass blooms from summer to fall. It can tolerate long periods of drought, so long as the sediment is wet. Its seeds and leaves are eaten by birds.

Chara vulgaris (muskgrass, stonewort) is an alga which grows in very hard water and is often calcified and brittle, due to the active precipitation of $CaCO3$ on cell walls. The plant is rooted (i.e. rhizomes), with its leaves (i.e. thalli) arranged in whorls. The plant grows completely underwater and has a distinctive musky smell (hence muskgrass). Nutrients and metals are neither taken up, nor translocated to the rhizomes, making them ideal for sediment stabilization. Stoneworts are branched multicellular algae that are often confused with submerged flowering plants. However, stoneworts have no flowers and will not extend above the water surface. *Nitella* has no odor and is soft to the touch, unlike *Chara,* which are light to dark green in color with forked, bushy branches, the branches appearing as whorls around the main stem.

Figure 6. *Chara vulgaris bed and Stukenia pectinata in BLACK CASTLE POND 3*

Stuckenia pectinata*** (Sago pondweed) is a perennial plant that arises from thickly matted rhizomes and has no floating leaves. The stems are thin, long and highly branching with leaves very thin and filament-like. The leaves grow in thick layers and originate from a sheath. The fruit is nut-like 1/8 to 1/4 inches long and 1/10 to 1/8 inches wide. It is an herbaceous plant up to 3 feet tall, generally completely submersed except for the reproductive stalk that peaks above the water. It flowers June through September. It is nearly un-branched at the base, becoming freely branched towards the top.

Sago pondweed occurs nearly worldwide and is found submerged in semi-permanent to permanently flooded areas where the water is less than 8 feet deep. It can be found from sea level to almost 16,000 feet above sea level. It can grow in nearly all bottom substrates and can tolerate high salinity, pH, and alkaline water.

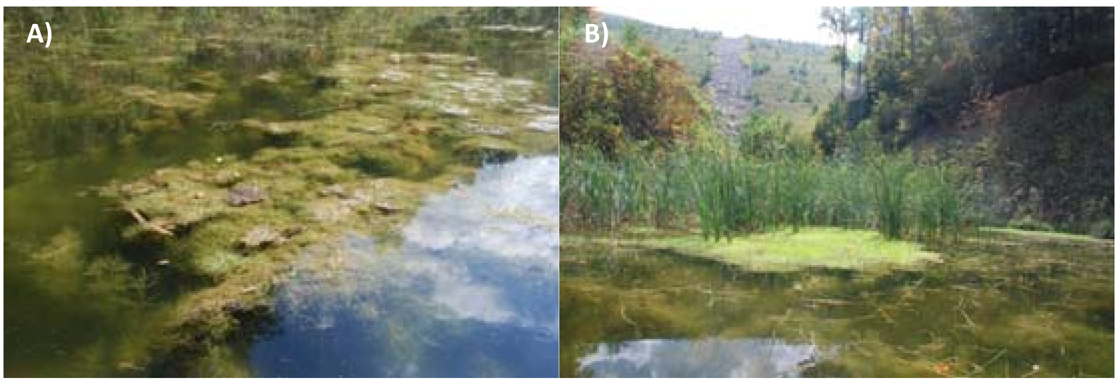


***Figure 7**. A)* Stukenia pectinata *and* Potemogeton pusillis *and B*) Luziola fluitans *and* Typha angustifolia *behind, from Cliffs-Dingus 1.*

Potamogeton pusillus (small pondweed) and ***Potamogeton foliosus*** (leafy pondweed). Both are emergent aquatic plants found in a wide range of habitats (small pondweed can tolerate brackish water, while leafy pondweed lives in marshes and shallow standing water), and widespread in the northern hemisphere. Both have fibrous roots, with and without rhizomes. Seeds and winter buds form at lateral branch tips and near leaf bases.

Typha angustifolia (*c*attail) is found in marshes, d itches, shorelines; shallow areas of lakes, ponds, and slow streams; quiet water up to 4 feet deep. It grows above water surface; is thickly rooted; has long, slender stalks growing 3 to 10 feet high; flower consists of a cigar-shaped "cattail", which is green during early summer and turns brown and fuzzy in the fall and following spring. Cattails help stabilize marshy borders of lakes and ponds; helps protect shorelines from wave erosion; northern pike may spawn along shore behind the cattail fringe; provides cover and nesting sites for waterfowl and marsh birds such as the red-winged blackbird; stalks and roots are eaten by muskrats and beavers; the starchy roots, young flowering spikes, and pollen can be eaten by humans, too.

Potamogeton natans (floating-leaved pondweed). This perennial pondweed has oval floating leaves and long, narrow, underwater leaves. The ribbon-like underwater leaves of ribbonleaf pondweed have a broad light green central stripe, and the floating leaves are often oppositely arranged. The underwater leaves of floating-leaved pondweed are so narrow they appear to be stiff leafless stalks, and the floating leaves often have slightly heart-shaped bases. The stem is generally unbranched, nearly cylindrical, to 2 mm thick. It has flowers in compact spikes less than 5 cm long on stalks to 12 cm long. Its fruit is produced in achenes. The root is fibrous and rhizotomous. It is distributed in ponds, lakes and slowly flowing water between 0.5 and 3 m deep throughout most of North America and Eurasia.

Potamogeton nodosus (longleaf pondweed) is a fully hardy perennial deciduous forb (non-grass, broad-leaved herb) with green flowers in midsummer. It grows well in semi-shade, and prefers high levels of water. This plant requires a minimum of 90 frost free days to grow successfully. It has no drought tolerance. *Potamogeton nodosus* grows in soils ranging from a pH of 5.8 (acidic ranges from 5.6 to 6) to 7 (neutral ranges from 6.6 to 7.5). It prefers medium fertility. It is a prostrate plant and has a rhizomatous growth form and has a fast vegetative spread rate.

Table 5. *Dominant vegetation cover estimates*

	Main % Cover by Taxon							
Site ID	**Chara**	**Potcri**	**Potfol**	**Potnat**	**Potnod**	**Potpus**	**Stupec**	**Typang**
BC_1	—	2	25	—	—	—	—	10
BC_2	2	—	—	—	—	—	20	—
BC_3	50	—	—	—	—	—	30	—
CF_1	—	1	—	60	5	1	20	10

Note: Black Castle Pond 1(BC_1), Black Castle Pond 2 (BC_2), Black Castle Pond 3 (BC_3) and Cliffs Pond (CF_1). Data from field trip #2 (June 2013).

Table 6. *List of major vegetation Latin and Common names*

Abbreviation	**Genus / species**	**Common name**
Chara	*Chara vulgaris*	common stonewort
Chara	*Chara vulgaris var. filiformis*	filiform stonewort
Luz	*Luziola fluitans*	water grass
Potfol	*Potamogeton foliosus*	leafy pondweed
Potnat	*Potamogeton natans*	floating-leaved pondweed
Potnod	*Potamogeton nodosus*	long-leaf pondweed
Potpus	*Potamogeton pusillus*	small pondweed
Stupec	*Stuckenia pectinata*	sago pondweed
Typang	*Typha angustifolia*	narrow-leaf cattail

The algal flora of the ponds is quite extensive. Even though *Chara* is an alga, it is considered a macrophyte, due to its large cells and growth form. Most of the algae were small, filamentous plants growing on rocks, or epiphytically on other vegetation. Some of the algae were single celled diatoms and blue-greens.

All of the algae collected from Black Castle Pond 1 were found after washing the vegetation samples taken. From these samples, 4 different blue-green algae were found: *Trachelomonas lacustris, Oscillatoria sp., Chroococcus dispersus, Microcystis sp.*. The same was true for Black Castle Pond 2. All of the washed algae were blue green algae, including *Closteriopsis sp., Chroococcus despersus, Tetraspora gelatinosa, Oscillatoria sp., Gleocapsa sp., and Phormidium sp.* Black Castle Pond 3 had another community of blue green algae, dominated by: *Microcystis sp., Oscillatoria sp., Leptolyngbya sp., Rivularia sp. Phormidium sp., and Chroococcus disperses*.

Cliffs Pond contained some floating mats of green algae composed mostly of *Spirogyra sp*. Washings from the vegetation produced many of the same algae as epiphytes: *Oscillatoria sp., Rivularia sp*., along with some green algae: *Mougeotia sp., Spirogyra sp.* and *Pandorina sp*. Several diatoms were also present, including: *Navicula sp., Rhizoclonium sp*., and *Tabellaria sp*.

On the 2nd field trip in 2013, we determined, not only the coverage of each of the major vegetation types, but took quadrat samples, as well. From these quadrants the density of the standing biomass of each of the vegetation components was determined (Table 7). With these values a total Se mass in the vegetation in the ponds per m^{-2} was determined. At each location (e.g. BC_1) and depth (e.g. 1.50m), we took a total of 256 g wet biomass from the sampled quadrat. The sample was entirely composed of *Potemogeton foliosus.* A subsample of the *Potemogeton* was dried, giving us dry biomass per quadrat, and therefore dry biomass per square meter. A second subsample was dried and shipped to the analytical laboratory at the University of Kentucky to determine the elemental composition. Multiplying the dry grams per square meter by the mg of selenium per Kg of dry mass, gave us the grams of selenium per square meter. Some of the vegetation found in the ponds as extrapolated from the biomass estimates contains a tremendous amount of selenium. For example, the *Stuckenia* in BC_2 was extrapolated to contain 2.8 grams of selenium per sq. meter of pond. In the Cliffs pond, the *Potemogeton* there also contained over 2 grams of selenium per square meter of pond.

Some aquatic vegetation are known hyperaccumulators of selenium, but, they can also translocate selenium from sediments up, into the submerged and aerial plant parts, possibly recycling selenium that has already been removed from the water. While we do not yet know the contribution of the *Potemogeton* or *Stukenia* to this process, we would not recommend them as a selenium removal system, since the Se can originate through the roots. Our primary focus has been on the alga, *Chara*, primarily because it has no root structure, merely an anchoring system. Nothing is transported from the sediment the biomass, but the biomass collects its elements from the water. All biomass decays at the sediment water interphase and grows at the top part of the biomass. Chara removed between 0.07 grams per square meter (sparsely colonized pond) to 0.9 g m^{-2} in more densely populated ponds.

Table 7. *Quadrat biomass data from June 2013 field trip.*

BIOMASS										
Site ID	Depth (m)	Species	Total wet grams	Dry g m^{-2}	Mn mg Kg^{-1}	Fe mg Kg^{-1}	Se mg Kg^{-1}	Grams Mn m^{-2}	Grams Fe m^{-2}	Grams Se m^{-2}
BC_1	1.50	Potfol	256	254.32	157.37	1564	1.97	40.0	398	0.50
BC_1	1.75	Potfol	57	45.2	157.37	1564	1.97	7.1	71	0.09
BC_1	0.50	Potfol	208	207.6	157.37	1564	1.97	32.7	325	0.41
BC_1	2.00	Potfol	96	92.16	157.37	1564	1.97	14.5	144	0.18
BC_1	1.50	Potfol	260	209.52	157.37	1564	1.97	33.0	328	0.41
BC_2	0.25	Stupec	90	126.56	1501.68	2262	3.24	190.1	286	0.41
BC_2	0.60	Stupec	390	703.36	1501.68	2262	3.24	1056.2	1591	2.28
BC_2	1.00	Stupec	370	431.84	1501.68	2262	3.24	648.5	977	1.40
BC_2	0.70	Stupec	440	855.76	1501.68	2262	3.24	1285.1	1935	2.77
BC_3	0.75	Chara	4450	304	2583.22	2313	2.92	785.3	703	0.89
BC_3	5.25	Chara	3560	202.72	2583.22	2313	2.92	523.7	469	0.62
BC_3	1.50	Chara & Stupec	5050	332.72	2448.00	2287	3.08	814.5	761	1.02
BC_3	5.50	Chara	40	23.84	2583.22	2313	2.92	61.6	55	0.07
BC_3	4.00	Stupec	6610	143.28	1501.68	2262	3.24	215.2	324	0.46
BC_3	0.50	Stupec	1470	168.24	1501.68	2262	3.24	252.6	380	0.55
CF_1	1.10	Stupec	57	3.68	n.s.	n.s.	n.s.			
CF_1	1.75	Potnat	170	185.12	128.45	817	9.43	23.8	151	1.75
CF_1	1.50	Stupec	227	254.96	n.s.	n.s.	n.s.			
CF_1	0.90	Potcri & Potus	312	367.76	71.67	198	6.29	26.4	73	2.31
CF_1	0.75	Potnod	57	55.36	n.s.	n.s.	n.s.			
CF_1	1.50	Potnat & Luz	142	118.88	128.45	817	9.43	15.3	97	1.12
CF_1	0.50	Stupec	28	10.56	n.s.	n.s.	n.s.			

= not sampled

Much of the vegetation sampled in the quadrats was covered in detritus or epiphytes. The epiphytes were smaller algae that grow on living surfaces. To see what impact these had on the selenium removal process, we washed samples of the vegetation that we collected. Any sediment, detritus, or loosely held material was washed into petri dishes, dried, weighed and sampled and analyzed. In Figure 8, the selenium concentration of the whole 'unwashed' vegetation is compared to the epiphytes or the material (detritus) washed off the plants. Generally, the 'washed' material contained more or a similar amount of selenium than the 'whole' vegetation on a dry weight basis. The *Chara* samples from pond Black Castle Pond 3 contained between 3 and 8 mg Se kg(dw)$^{-1}$, while the epiphytes on the *Chara* contained similar concentrations of selenium, between 3.8 and 7 mg Se kg(dw)$^{-1}$. Because the epiphytes are a small weight percentage of the 'whole', their overall contribution to selenium removal is small. However, if Chara is covering the sediment bottom extensively larger quantities of epiphytic material is collected on the biomass and relegated to the sediment with the Chara.

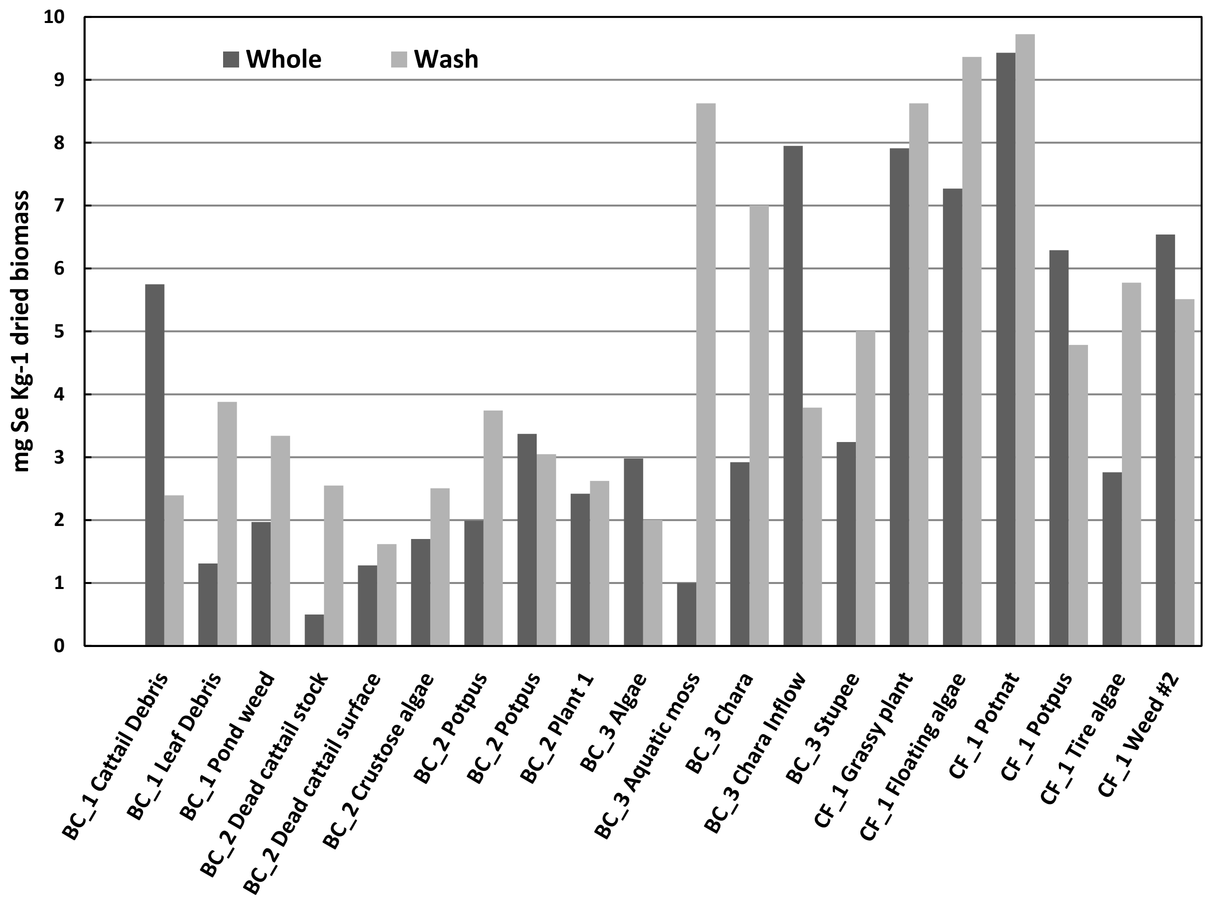


Figure 8. *Comparison of 'whole' and 'washed' samples for selenium.*

3.3 Water Chemistry

All of the ponds visited in October 2012 and June 2013 had pHs within the neutral range from 7 to just over 8 units (see Tables 8 and 9). Conductivities, a measure of the total dissolved solids, were relatively high, ranging from a low in the Cliffs Pond (1,400 µS cm^{-1}), to a high in Black Castle Pond 3 of 2,600 µS.cm^{-1}. These high conductivities reflect the relatively high concentrations of calcium, magnesium and sodium – all present in harder waters. Selenium concentrations in all of the ponds, from the two field trips, ranged from a high of 28 µg.L^{-1} in the Cliffs Pond (October 2012 field trip) to a low of 9.5 µg L^{-1} in the effluent of Black Castle Pond 2 (June 2013 field trip). The introduced biomass grew in water with an order of magnitude lower electrical conductivity in Indiana as was measured in the valley fill ponds.

Water sampled on the October 2012 from BC and Cliffs ponds contained about 32 mg L^{-1} nitrate and another 15 mg L^{-1} nitrite (Table 10) and from the second field trip (Appendix 1, page 2). These concentrations are high and provide adequate nitrogen for the growth of aquatic vegetation. The phosphate concentrations on the other hand for the 2013 collection are all below detection limit, which is 0.9 mg L^{-1}. A concentration of 32 mg L^{-1} nitrate is equivalent to 533 µM nitrate (very high for aquatic systems). If we take the detection limit value of 0.9 mg L^{-1} as the phosphate concentrations then this is equivalent to 9 µM, it would result in a molar ratio between the two elements of around 60, which is 4x that of an ideal N:P ratio. It may suggest that with additional phosphate, even more biomass could be produced, or presently phosphate might be limiting.

Table 8. *Field water chemistry from Trip #1*

Site	pH	Conductivity (µS cm^{-1})
BC_1 inflow	7.27	2,230
BC_1 outflow	8.03	1,460
BC_2 inflow	7.27	2,380
BC_2 inflow	7.38	2,390
BC_3 inflow	7.51	2,590
BC_3 outflow	7.37	2,470
CF_1 inflow	8.27	1,290
CF_1 outflow	8.08	1,380

Black Castle Pond 1 (BC_1), Black Castle Pond 2 (BC_2),

Black Castle Pond 3 (BC_3), and Cliffs Pond (CF_1) for October 2012.

Table 9*.Field water chemistry from Trip #2 (June 2013). Site abbreviations as in Table 8.*

Site	Sample	Temp (°C)	pH	Conductivity (µS cm^{-1})
BC_1	Surface	18.9	7.31	2030
BC_2	Surface	18.8	7.33	3310
BC_2	Surface(Floc)	18.8	7.64	3230
BC_3	Surface	18.9	7.74	3240
CF_1	Surface	18.9	7.66	1690

__Table 10.__ Water chemistry from field trip #1(UK Environmental Chemistry Lab).

Analysis Code	Fe ($mg.L^{-1}$)	Se ($\mu g.L^{-1}$)	F^- ($mg.L^{-1}$)	Cl^- ($mg.L^{-1}$)	NO^-_2 ($mg.L^{-1}$)	SO_4^{2-} ($mg.L^{-1}$)	Br^- ($mg.L^{-1}$)
Detection limit	0.01	0.01					
BC_1	0.033	15	n.a.	13.9	15.1	1780.3	BDL
BC_2W							
BC_3	0.008	28	n.a.	3.9	13.3	1748.4	BDL
Analysis Code	NO_3^- ($mg\ L^{-1}$)	PO_4^- ($mg\ L^{-1}$)	Na^+ ($mg\ L^{-1}$)	Mg^{2+} ($mg\ L^{-1}$)	Al^{3+} ($mg\ L^{-1}$)	K^+ ($mg\ L^{-1}$)	Ca^{2+} ($mg\ L^{-1}$)
Detection limit							
BC_1	32	BDL	18.7	382.3	0	28.7	299.6
BC_2W							
BC_3	34.4	BDL	15.9	362	0	27.2	290.6

Note: units of other elements but Se in $mg.L^{-1}$

Based on our final report from ARIES 1, we felt that some of the selenium in the valley fill effluents was present as particulates or nano-particulates. As a rough first cut at this hypothesis, we had the water samples analyzed 'whole' and after passing through a 0.2µ filter. In Appendix 1 the differences in concentrations can be examined for all elements in the filtered 0.2µ and the unfiltered water samples referred to as total recoverable element (pages 3-12). The differences for selenium are within 1 $\mu g.L^{-1}$ or even negative, with one exception (likely an error). One would expect to be able to determine particulate selenium by subtracting the amounts passing through a 0.2µ filter from the amounts retained. But, according to EPA method 200.8, this is not the case as the total recoverable Se concentrations would have to be digested.

> "For the determination of total recoverable analytes in aqueous and solid samples a digestion/extraction is required prior to analysis when the elements are not in solution (e.g., soils, sludges, sediments and aqueous samples that may contain particulate and suspended solids). Aqueous samples containing suspended or particulate material 1% (w/v) should be extracted as a solid type sample (Section 11.2.2)." http://www.caslab.com/EPA-Methods/PDF/200_8.pdf

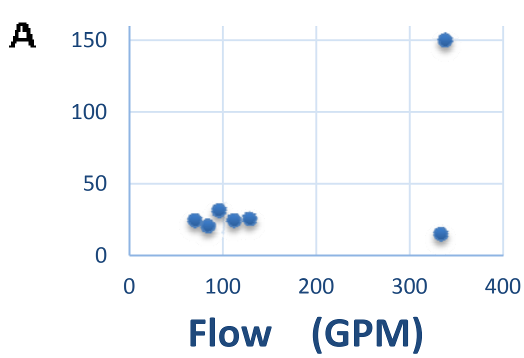
Therefore, given that these digestions / extractions for suspended solids were not performed, we can only speculate on the particulate composition of the valley fill selenium. This is unfortunate and should be evaluated during the pilot test phase.

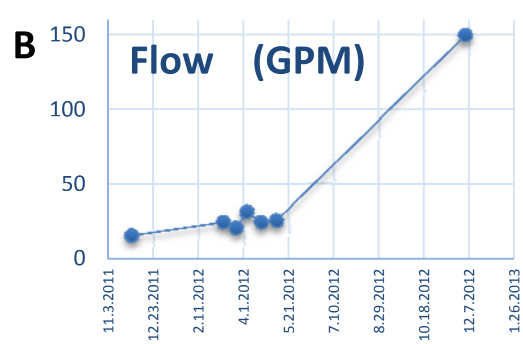
3.4 Selenium Loading

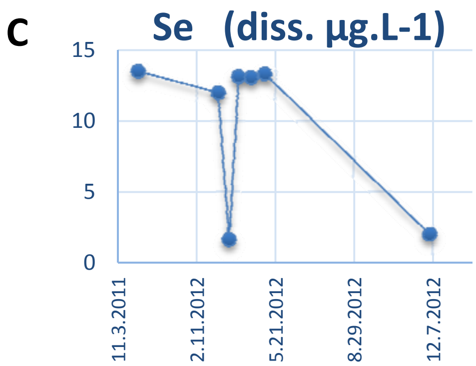
Selenium loadings are defined as the mass of selenium flowing through the ponds on an annual basis. To calculate loadings, we multiplied the selenium concentration in the water by the flow (gpm) of water entering the valley fill. Since we do not have any data for flow rates entering the valley fills, we assume that the volume of water entering the valley fills equals the water exiting the valley fills. This assumption is only true if there is no water entering or leaving except through the official monitoring station. Our calculations of loadings are thus dependent on the number of effluent flow rate samples taken per year. To somewhat compensate when few flow data are given for a site, we defined a sampling period as the interval between flow measurements. This can vary from weekly to nearly a whole year. Pond Black Castle Pond 3 has no flow data and is connected to Black Castle Pond 2 into which it flows. Black Castle pond 1 is completely separate from the other two ponds.

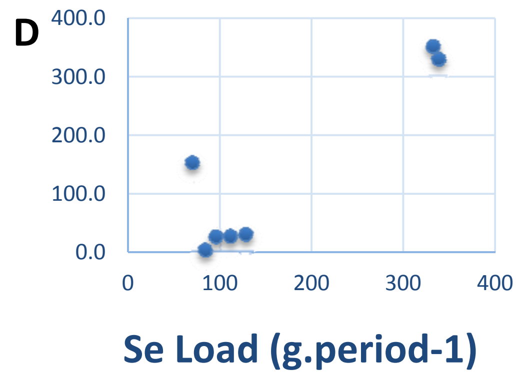
For example, for BC-2 pond (monitoring station VW1012441) we have only one datum point for 2011, thus representing the entire period of 365 days. In 2012, periods ranged from 210 days to as few as 12 days. With so few data points, the loadings per period become very unrealistic. The range of flow values is also somewhat dramatic with values around 15 GPM to 150 GPM. It appears as if there is a store of clean water in the valley fill which is followed by water with about 14 µg L^{-1} Se, which passes through the outflow during storm events. It indicates complex hydrological conditions in the drainage basin feeding the pond. For example, at sampling station WV1012441 (Black Castle Pond 2) there we were only provided with 7 flow measurements over the last 3 years. These are plotted in Figures 9A-D. If we extrapolate each day for which we have measurements to the next day we have measurements, and sum these over the year, we can estimate an annual loading. For WV1012441, the loadings are 2011 (n =1; 351 g Se $year^{-1}$), 2012 (n=6; 563 g Se $year^{-1}$). These loadings of Se are certainly prone to error, given the few values used.

Figure 9. A-D: *Pond Black Castle Pond 2 monitoring data WV1012441 (Black Castle Pond 2 outflow, Lower of the two ponds in series and also called East of Stallings on drawings)*









For most of the year, flow was reasonably steady between 15 and 31 GPM (Figure 9A). The only major difference came in a single rain event in November of 2011 with 150 GPM. However, if the flows are plotted on in a linear time sequence (B), it appears as if flows are steadily increasing. The selenium at that outlet varied between 2 and 13.3 µg L^{-1} from November of 2011 to the end of 2012 (Figure 9C). With so few data points no seasonal trends are evident, but it appears that high flows result in low Se concentration.

Figure 10A (WV 1020358) BC-1 pond shows the effluent flow (Outlet 024) on a seasonal basis (plotted as day of year). Winter months may have slightly higher flows than summer, with the exception of a couple of rain events in January and February 2013. These are shown in Figure 10B. Flows varied from 12 GPM (April 2012) to a high of 200 GPM (February 2013). Over the same period, the selenium concentrations (Figure 10C) at that outlet varied between 0.24 (May 2012) and 38.8 µg L^{-1} (April of 2012). By multiplying the flow by the selenium concentration, we calculated the Se load. These data are presented in Figure 10D. Loads varied between 18 g d^{-1} to a low of 0.03 g d^{-1} (data not shown) or 0.4 to 2286 g Se $period^{-1}$(Figure 10D) and for the year the we estimate an annual loading for 2011 (n=1; 2286 g Se $year^{-1}$), 2012 (n=7; 1350 g Se $year^{-1}$), and so far this year (n=6; through February – 891 g Se $year^{-1}$). In summary these **loadings are not reliable at** all in the absence of regular measurements. Essentially, these considerations demonstrate that in order to arrive at the effectiveness of a treatment system flow in and out need to be monitored.

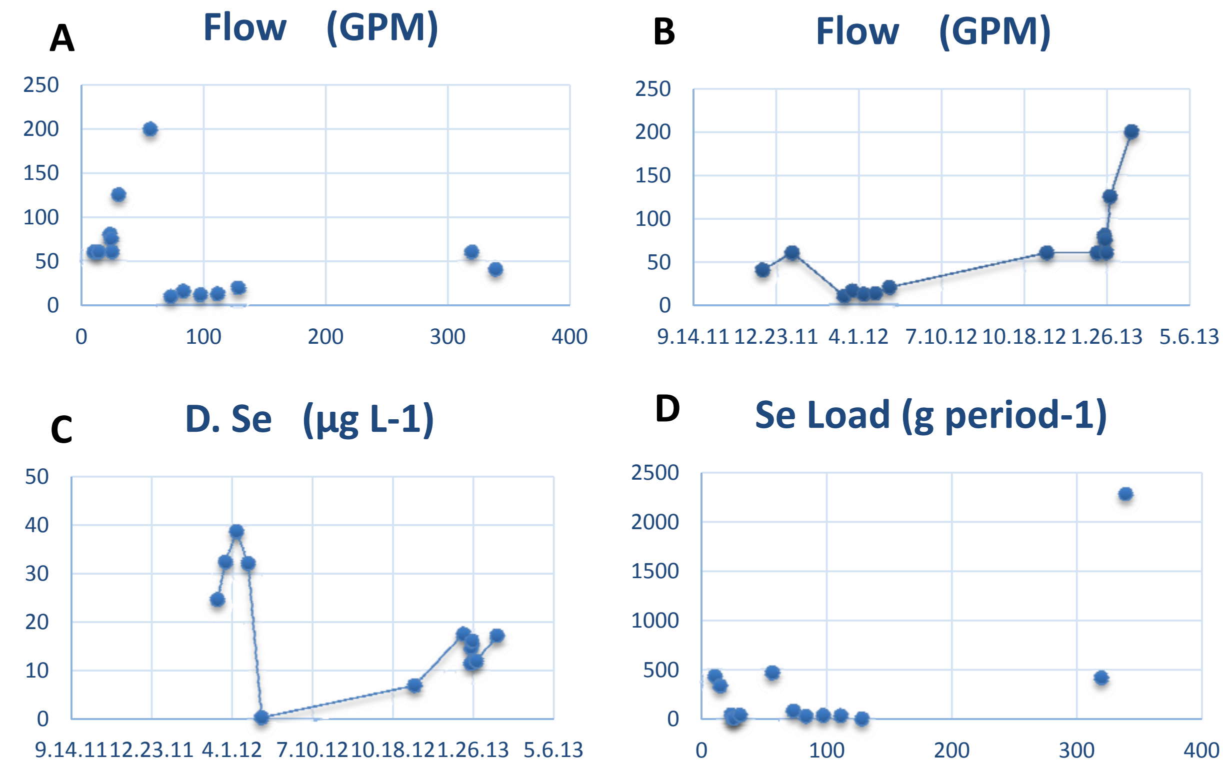


Figure 10. *A-D: Black Castle Pond 1 (WV1020358, Called locally Morgan's*

The monitoring data for the **Cliffs Pond come from WV1016750** monitoring station (Figure 11, A-E) is the best dataset available. Data ranged from January 2010 to January 2013. Flow (A) varied from 5 GPM (October 2010) to a high of 584 GPM (January 2013 data not shown as it was an outlier). Over the same period, the selenium concentrations Figure 11 B at that outlet varied between 0.5 (April 2012) and 42 µg L^{-1} (October of 2010). From the date graph (A), flows were definitely higher in 2012 and 2013 than in 2010. Selenium, however, seems to be decreasing with time (B). In graph C, we plotted flow vs. selenium concentration. The data are clumped heavily at the low flow end. Higher selenium concentrations are associated with lower flows. This suggests that precipitation events bring freshwater into the watershed, diluting the existing effluent. The selenium load is shown in Figure 11D. Loads varied between 27 g d^{-1} to a low of 0.07 g d^{-1} resulting in annual loadings for 2010 (1090 g Se year^{-1}), 2011 (1680 g Se year^{-1}), 2012 (1680 Se year^{-1}), and so far this year (through February – 960 g Se year^{-1}). Since there were no data during 2011, we took two year total and divided by 2. The loadings peak during summer months and are low during winter months. Finally, we plotted selenium concentrations and flow against day of year (11E) to look for seasonal patterns and as expected t selenium concentrations are higher when flows are lower.

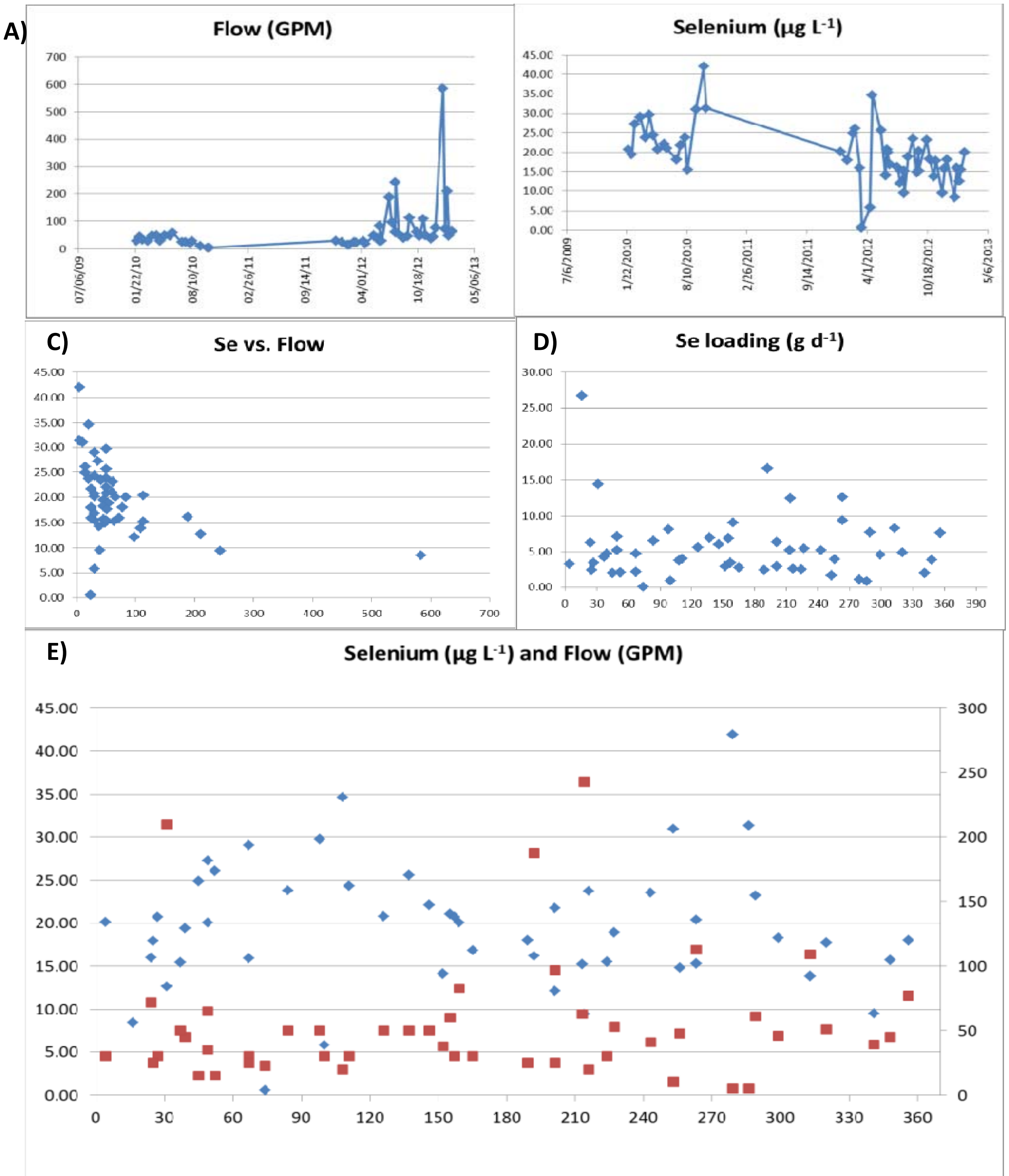


__Figure 11.__ Monitoring data from Cliffs Resources. A) Flow measurements by date taken at sampling station (WV1016750). B) Selenium concentrations at the monitoring station by date. C) Selenium concentration graphed against flow rate. D) Selenium loading (g d-1) plotted by day of year. E) Selenium concentration (left axis; µg L-1) and flow (right axis; GPM) plotted by day of year.

We do not know if these monitoring data are assigned to the correct locations but given the large data gaps, this entire section serves as examples what would be needed to assess treatment effectiveness**.** In summary these very rough estimates ranged between 0.5 kg to 2.3 kg per year for all ponds.

3.5 Sediments as selenium sinks

Two sediment cores were obtained during the June 2013 field trip, from the Cliffs pond (surface to 10 cm) and Black Castle Pond 1 (Appendix 1, pages 14-21). The highest selenium concentrations were found in the surface horizon (0-2 cm)of Cliffs pond core 17 $\mu g.g^{-1}$, decreasing to 7 $\mu g.g^{-1}$ at a depth of 2-4 cm with a further decrease to 1.37 $\mu g.g^{-1}$ in the next 2 cm segment and finally below 6 cm, concentration of 0.6 $\mu g.g^{-1}$ was found. The detection limit is 1.7 $\mu g.g^{-1}$ and a minimal analytical error of about 1 $\mu g.g^{-1}$. The decrease of selenium with depth demonstrates the accumulation of selenium in the pond sediment.

Unfortunately, the second core was collected in Black Castle Pond 1. This pond was dredged in 2012 and hence it does not show any vertical trends in Se concentration. The highest concentration of selenium in the sediment core samples was 1.5 $\mu g.g^{-1}$ at a depth of 20 cm. All other samples had concentrations just below 1 $\mu g.g^{-1}$.

3.6 Projections

Given the wide range of Se loading estimated we used *Chara* biomass to assess if the algae could indeed remove sufficient Se with its growth if it were present in the ponds using an annual growth rate per year based on our previous mine waste water projects.

Black Castle Pond 3 was the best pond for *Chara* growth. It sits above Black Castle Pond 2, so water draining from Black Castle Pond 3, enters Black Castle Pond 2. *Chara* covered 50% of the surface area of bottom sediments (Table 1). From this pond, we collected 3 samples (304, 202, and 24 dry grams m^{-2}; see Table7). The average was 177 dry grams of *Chara* per square meter. Since Black Castle Pond 3 is 0.58 ha in surface area (see Table 1), and *Chara* covers 50% of the area (see Table 1), *Chara* covers 0.29 ha or app. 2900 m^2. Multiply the 177 dry grams m^{-2} x 2900 m^2, gives app. 514 kg of dry *Chara* mass in the pond. Since the *Chara* population sampled from Black Castle Pond 3 contained between 2.9 and 8.0 mg of Se per kg (see Table 11), we can multiply the total biomass in the pond by the selenium concentration. Thus the standing biomass of 514 kg contained approximately 1.5 kg of selenium on the low side and about 4.1 kg of selenium on the high side. However, we do not know how long this biomass has been growing. To estimate how much selenium could be removed on an on-going basis, we need to factor in the growth rate of the *Chara*.

We were not able to measure any site specific growth rates of *Chara*, because the transplanted algae were not found on the second field trip. On the last field trip *Chara* biomass from BC-3 has been placed in nettings and site specific growth rates can be obtained in 2014 if desired.

Growth rates of *Chara* are common in the literature and available from previous studies performed by Boojum Research in other mine effluents. To simplify the projections, we have used conservative growth rates which around 100 g (dw) $m^2.year^{-1}$. With a growth rate of 100 g(dw) $m^2.year^{-1}$ applied to Black Castle Pond 3 data gives 0.1 kg dry mass per square meter x 2904 sq meters [area of *Chara* in pond] = 290 kg(dry).$m^{-2}.year^{-1}$. At 1.5 mg of selenium per kg, that results in 0.44 g Se removed per year. At the high end, 8.0 mg of Se per kg, multiplied by 290 kg (dw).$year^{-1}$ gives 2.3 kg of Se removed per year. This suggests that the populations may be 2-3 years old.

Calculations of Se loadings for Black Castle Pond 3 were quite variable and sparse. Based on the information provided (2011 = 1.06 g $Se.d^{-1}$; 2012= 1.50, 0.17, 2.12, 1.64, 1.74, 1.56 g $Se.d^{-1}$), the Se loadings were estimated to be 0.35 kg Se $year^{-1}$ in 2011, 0.56 kg.$year^{-1}$ (2012, n=6). If these numbers are in the 'ball park' for loadings, then the existing *Chara* populations should be able to sequester the selenium loadings. Since the removal is predicated on growth, we should see significantly lower selenium concentrations leaving Black Castle Pond 3 in the summer. We could not determine this, though, as there were no inflow pond inflow concentration numbers obtained. *Chara* growth rates are lower in the winter, but still positive. With enough biomass in the pond, it is conceivable to produce significantly lower selenium concentrations year round. In fact, Scott Perdue, from Black Castle, reported to our Dr. Scribailo that recent selenium concentrations leaving Black Castle Pond 3 were below detection limits. We can only speculate that the selenium numbers and abundant *Chara* populations in Black Castle Pond 3 are correlated.

.

3.7 Other Algae and Vegetation as Concentrators

In Table 11 we present selenium concentrations from vegetation, it's parts, debris and water samples from the three valley fill ponds. Black Castle Pond 1 cattails roots contain 2.5 $\mu g.g^{-1}$ Se, Black Castle Pond 2 cattail roots contain 54 $\mu g.g^{-1}$ Se, while the rhizomes and the leaves contained only 5 $\mu g.g^{-1}$, suggesting that the root might have been digested with the adhered sediment. A bottom covering algae in Cliff's pond contained 41 $\mu g.g^{-1}$ Se. In short, selenium is in every component of the pond ecosystem. The growth and decay of these plants will contribute to the selenium concentration in the water, to what degree is not known.

We have calculated the 'concentration factor' or 'concentration of selenium in plant material / concentration in water.' From the ARIES I literature summary it became evident that number of plants are referred to as hyper-accumulating selenium and some plants, such as *Chara* have been used in the removal of selenium from industrial effluents. A large fraction of the literature however refers to soil and their growth use if harvested reduces soil Se concentrations. However if the biomass decays in the water, and mobilizes the Se from the pond sediment, this is not desirable. The question is evident, are some of the plants present in the valley fills from Black Castle mines and Cliffs mines are hyper-accumulators? Those plants which have concentration factors over 1000 are considered hyper-accumulators. From Table 11, we can see that *Scirpus* roots, *Chara*, *Potemogeton pusillus*, cattail roots, some unidentified algae, *Stukenia* and several unidentified samples. The list is quite long. Essentially the colonization of these plants is not desirable and an investigation into their function in relation to Se cycling in the ponds is also futile, as they colonized the ponds spontaneously. Characean populations, if allowed to colonize the ponds first, will likely dominate the ponds for a considerable length of time. Their dominant pioneering ecological status is well documented for disturbed lakes and ponds. We also analyzed the biomass for a number of other elements to see if there were any associations with selenium in vegetation, as would be expected with sulphur. These data can be found in the Appendix 1 (pages 18-23).

***Table 11**. Concentration factors of selenium in tissues collected in ARIES II field trip*

Sample	[Se] $\mu g.g^{-1}$ in tissue	[Se] $\mu g\ L^{-1}$ in water	Dry(g)/ Wet(g)	Conc. Factor
BC_1	0.87	29.83	0.181	161*
BC_1 Before S. Curtain	0.88	29.83	0.181	163
BC_1 Cattail root	2.52	29.83	0.181	468
BC_1 Cattail- Rhizo	0.52	29.83	0.181	96
BC_1 Cattail- leaf	0.50	29.83	0.181	93
BC_1 Pond weed	1.97	29.83	0.181	365
BC_1 Leaf debris	1.31	29.83	0.181	243
BC_1 Float cattail debris	5.75	29.83	0.181	1065
BC_1 Scripus root	5.74	29.83	0.181	1063
BC_1 Scripus leaf	2.47	29.83	0.181	457
BC_2 Plant 1	2.42	10.9	0.181	1227
BC_2 *Chara*	3.57	10.9	0.181	1810
BC_2 Potpus	1.99	10.9	0.181	1009
BC_2 Nearshore crust. algae	1.70	10.9	0.181	862
BC_2 Potpus	3.37	10.9	0.181	1708
BC_2 Cattail stock, dead	0.50	10.9	0.181	253
BC_2 Cattail surface, dead	1.28	10.9	0.181	649
BC_2 Cattail root	54.25	10.9	0.181	27495

Sample	[Se] µg.g^{-1} in tissue	[Se] µg L^{-1} in water	Dry(g)/ Wet(g)	Conc. Factor
BC_2 Cattail Rhizo	5.76	10.9	0.181	2917
BC_2 Cattail leaf	5.02	10.9	0.181	2544
BC_3 Algae	2.98	11.1	0.181	1483
BC_3 Chara Inflow	7.95	11.1	0.181	3957
BC_3 Aquatic moss	1.00	11.1	0.181	498
BC_3 *Chara*	2.92	11.1	0.181	1453
BC_3 Stupee	3.24	11.1	0.181	1613
CF_1 Potpus	6.29	22.8	0.181	1524
CF_1 Grassy plant	7.91	22.8	0.181	1917
CF_1 Weed #2	6.54	22.8	0.181	1585
CF_1 Potnat	9.43	22.8	0.181	2285
CF_1 Algae bottom	41.33	22.8	0.181	10015
CF_1 Floating algae	7.27	22.8	0.181	1762
CF_1 Tire algae	2.76	22.8	0.181	669
CF_1 Root	4.84	22.8	0.181	1173
CF_1 Leaf	13.69	22.8	0.181	3317
CF_1 Plant Root	1.87	22.8	0.181	453
CF_1 Leaf	29.10	22.8	0.181	7051

* (870/29.82)/0.181 = 161

** highlighted red values are considered hyperaccumulators

Note: (dry/wet) ratios are averages of ARIES I data. Note that the pond abbreviations are the same as described in Table 8.**

The concentrations factors vary between 27,000 (cattail roots) and less than 100 times when water and plant concentrations are compared. One can easily see that within one plant different parts concentrate differently and decaying material can be associated with a considerable mass of selenium. The vegetation (and vegetation parts) analyzed concentrated selenium. In rooted vegetation, this is reasonable, since the selenium was most likely taken up from the sediments into the other plant structures. Part of the roots and the rhizomes are often perennial, i.e. grow over a number of years, but would ultimately decay when the root-mass chokes itself. In summary the information in Table 11 highlights, that generally the vegetation may be problematic with respect to the selenium concentration in the water.

The growth of charophytes is different – they take nutrients from the water up through their large cell walls and on the outside of the cell walls over the entire biomass. They have no roots, but are simply anchored in the sediments. Cell walls mineralize metals through specialized physiology, having on the outside acidic and alkaline regions. Their growth form is apical growth and basal decay, which means the plants grow from the top and degrade from the bottom, while sinking into the sediment. In addition, the biomass collects debris and TSS on its surface, which contributes to water clarity. These are all well documented characteristics of charophytes and make them primary colonizers of disturbed sites and ideal candidates for biological polishing in valley fill ponds.

4.0 Conclusions

In the introduction we set up a decision tree to aid us in understanding how to effectively use the pond systems as a treatment system. We needed to assess roughly the "pathway "of the selenium in the pond ecosystem. Although these questions were useful, we could not fully answer all of them. The answers are given in italics.

Is the incoming water higher, lower or unchanged in [Se] than the water leaving the pond? *Detailed discussion of the investigated ponds below.*

- If the outflow [Se] is **higher** than the inflow [Se] :
    - then could the sediments be the source? ***Yes***
    - then could the decaying plants debris be the source? ***Yes***
    - or could the [Se] come from a different additional water source? ***Do not know***
- If the outflow [Se] is **lower** than the inflow [Se]:
    - is the Se carried by the debris to the sediment? ***Do not know***
    - is the Se in the perennial roots of the rooted plants? ***Yes***
    - Is the Se in the annual parts of the rooted plants? ***Yes***
    - is the Se on the epiphyton, which might be liberated to the water by waves? ***Yes***
- If the outflow selenium concentration is unchanged when the water is leaving the pond:
    - Are there any obvious differences to the other two described conditions. ***Yes***
    -

How do selenium and other elemental concentrations differ between the inflow and outflow?

- **the [Se] is unchanged between inflow and outflow but other elemental concentrations are leaving the pond at higher concentrations than at the inflow :**
    - ***Cliffs Pond***: The selenium concentrations are similar to those leaving the pond, but sulfate, sulfur, nitrate, magnesium and manganese concentrations are higher in concentrations at the outflow of the pond. Intuitively these increases are due to the extensive free-floating algal populations and the vegetation in the pond.
        - This pond is extensively vegetated and therefore the sediments do accumulate and integrate selenium, as was shown by the sediment core data.

- **Could the decaying plant debris be the source?** *Very likely. The Cliffs pond contained a great deal of vegetation, much of which may not be integrated into the sediment.*

- ***Black Castle Pond 2***: This pond has essentially the same selenium concentration entering the pond as reporting to the outflow. However, the sulfur concentrations were remarkably lower in the outflow, which might be due to Chara, as they are high on sulphur.

➢ **[Se] and other elements are lower at the outflow than inflow**:

- **Black Castle Pond 3:** Selenium concentrations are remarkably lower than the inflow reporting a reduction of 7 µg L^{-1}. Similarly, sulfur, sulfate, vanadium and magnesium are also leaving the pond at lower concentrations than were reported for the inflow sample.
  - **Is the selenium carried by the debris to the sediment?** *We do not have a reliable sediment core from this pond, but it is suggested from the core taken at Cliff's pond.*
  -

In order to relate the reductions of the Se concentrations to the charophytes it would be relevant to determine the selenium concentration and the amount of water moving though BC 3 pond and entering from the side over the intensely encrusted wall. This wall brings water into the pond the volume, quality and the Se concentration we do not know. It could well be together with the presence of extensive *Chara* growth the cause of the reduction in selenium as well as sulfur and sulfate. The sulfur concentrations in the *Chara* range between 0.28 to 0.82 %. According to Ralston *et al*. (2008) and many other bio-geochemists, selenium and sulfur behave in a geochemically-similar manner. Therefore, the reduction of sulfur (not the sulfate) could well be related to the selenium concentration reductions. Although it is at present only a speculation, both Black Castle Pond 2 and Black Castle Pond 3 show reductions in sulfur by about 1000 mg L^{-1} between inflow and outflow, and both have *Chara* populations.

➢ **The outflow [Se] is unchanged when the water is leaving the pond:**

- ***Black Castle Pond 1:*** The selenium and most other elemental concentrations are relatively unchanged passing through Black Castle Pond 1. The only exceptions might be barium and iron. The samples taken close to a sediment curtain were used for comparison.
  - **Are there any obvious differences to the other ponds?**
    This pond was dredged in 2012 is the least vegetated and has a disturbed sediment. Chara biomass should be added to this pond along with sediment curtains to reduce the suspended solids. It could be considered as a pilot test system but this incoming water over the wall and the hydrological conditions should be assessed.

4.1 Objectives achieved?

Our section of the ARIES II project had 6 objectives:

1. **Acquire design parameters for selective sediment ponds at Industrial Affiliates mining operations to ascertain Se removal by plants, algae and microbes.**

   We sent field teams to industrial affiliate sites in the fall of 2012 and again in the late spring of 2013 with the express purpose of collecting design parameters. Field teams collected plants, algae and microbes for later selenium and elemental analyses. They also sampled water and sediments for further laboratory analyses.

2. **Placement/establishment of algae at selected Industrial Affiliate mines.**

   We sent a field team in the fall to transplant *Chara* and *Ceratophyllum* to industrial affiliate operations. First transplants failed for unknown reasons. Plants were not recovered in the spring.

3. **Participate in sampling.**

   We subcontracted our field trips to a professor of lake biology from Purdue University North Central (Dr. Robin Scribailo). He and his colleagues collected specimens of the biota from 3 Black Castle Valley Fills (Black Castle Pond 1 [WV1020358]**,** Called locally Trout Pond and also Morgan's Branch, Black Castle Pond 2 [WV1013441] the lower of the two ponds in series and also called East of Stallings on drawings, Black Castle Pond 3 the upper of the two ponds in series also called East of Stallings on drawings, and Cliffs-Dingus Pond 1 (WV1016750). These three ponds are described in detail above. The above report details our report on the hydrology, biology and chemistry of these 4 ponds. We have extensive samples from two sample dates (October 2012 and June 2013). Samples have been preserved at the Purdue University North Central and would be available for further analysis if desired.

4. **Biomass identification and classification.**

   Included in the report is a section on the dominant algae, vegetation found in these 4 ponds.

5. **Analysis of biomass**.

   Our field teams measured the standing biomass of the dominant vegetation, including the alga *Chara*, and the dominant aquatic vegetation types. Since our transplanting efforts did not produce any viable populations, we could not measure overwintering growth rates. Many metals and other elemental concentrations were determined for the biomass, thus a good database specific for the ponds exists for further interpretation, if desired.

6. **Participate in assessment of design parameters**

   Design of a biological system for the removal of selenium requires several components.

1. It requires a pond in which biota with a suitable growth form exists such as charophytes. Its growth needs to be promoted through ecological engineering measures (such as seeding or transplanting) such that it self-perpetuates as the dominant vegetation. We found charophytes naturally colonizing 2 of the 4 ponds.
2. It requires charophytes to remove (adsorb, absorb, and or volatilize) the selenium which occurs in the effluent. We determined that this is the case.
3. It requires a long enough residence time so that selenium has a chance to contact biota as it passes through the pond. We do not know to date the residence time as we do not know the inflow rate nor the [Se] concentration coming into of the ponds , only the outflow rate and its [Se].
4. Charophyte populations provide a selenium sink as their biomass retain selenium until they reach the sediments, and with the population provides a sediment cover ensuring that the sediment remains reducing.

We have completed, with this report, all 6 of the objectives laid out in ARIES II Subtask 2.2.3. We have provided enough information for the industrial affiliates to make a decision as to the efficacy of the program and generated enough interest that they might want to move forward with an ecological-engineering approach to selenium removal in selected valley fill ponds.

5.0 References

Prescott, G. W. 1962. Algae of the Western Great Lakes Area. 2nd edition. Wm. C. Brown Company, Dubuque, Iowa.

Prescott, G. W. 1978. How to Know the Freshwater Algae. 3rd edition. WCB McGraw-Hill, New York.

Ralston, N.V.C., Unrine, J., and Wallschläger, D. 2008. Biogeochemistry and Analysis of Selenium and its Species. North American Metals Council, Washington, D.C.

Thorp, J.H. and A.P. Covich (eds.) 2010. Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego.

Wehr, J.D. & Sheath, R.G. (eds.) 2003. Freshwater Algae of North America: Ecology and Classification (24 chapters), Academic Press, San Diego.

Ziemkiewicz, P.F. & Lovett, R.J. 2011. Natural selenium attenuation at the lab, outlet and watershed scales. West Virginia Mine Drainage Taskforce Workshop . 2011

APPENDIX 1 : Raw data

Graphics sorting high to low concentrations

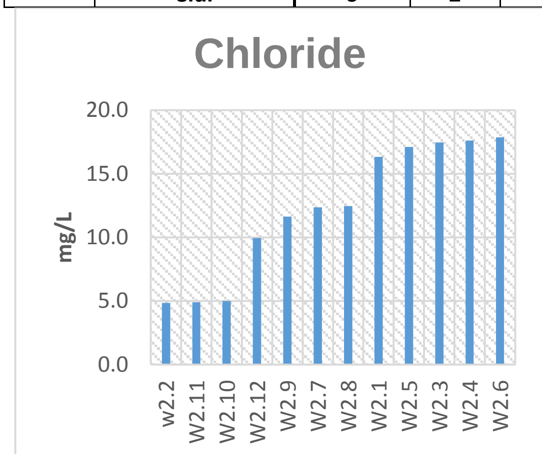
Table of contents

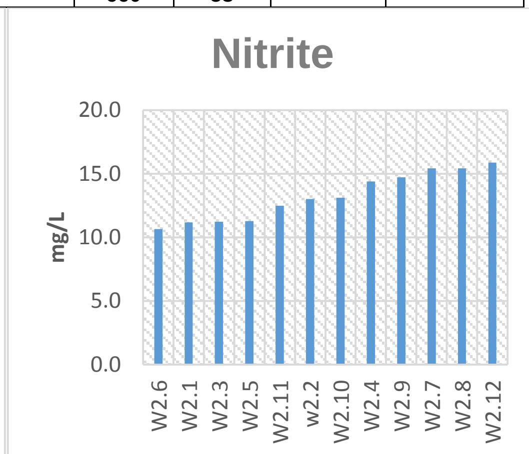
Note: The elemental composition of the biotic component of the vegetation and debris has been collected in part for also for future investigation to assess elemental cycling and ecological behavior of the ponds. A full interpretation of these data would be carried out during a field pilot study of the proposed treatment option.

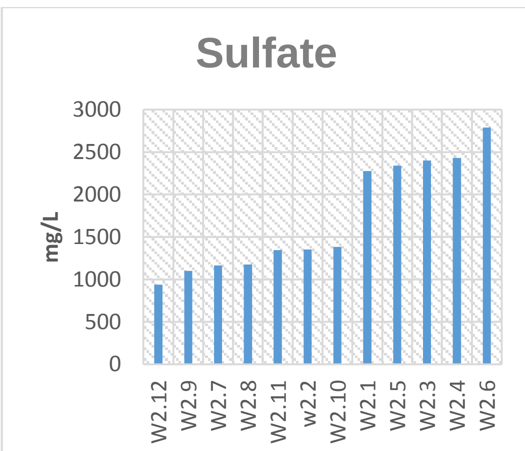
1. Concentrations of Cl, NO_2, F, SO_4, NO_3, Br,

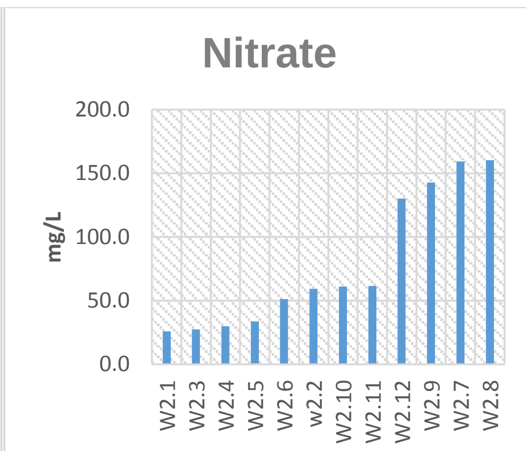
mg/L

		Chloride	Nitrite	Flouride	Sulfate	Nitrate	Bromide	Phosphate
b	BC #2 outflow	16.3	11.2	BDL	2275	25.8	BDL	BDL
w2.2	BC 1 outflow	4.8	13.0	BDL	1352	59.4	BDL	BDL
W2.3	BC 2	17.5	11.2	BDL	2401	27.3	BDL	BDL
W2.4	BC 2 inflow	17.6	14.4	BDL	2433	29.8	BDL	BDL
W2.5	BC 3	17.1	11.3	BDL	2341	33.8	BDL	BDL
W2.6	BC 3 inflow	17.9	10.7	BDL	2789	51.2	BDL	BDL
W2.7	Cliff Pond #28	12.4	15.4	BDL	1164	159.3	BDL	BDL
W2.8	Cliff Pond #28 rep	12.5	15.4	BDL	1174	160.2	BDL	BDL
W2.9	cliff pond outflow	11.6	14.7	BDL	1101	142.6	BDL	BDL
W2.10	H20 BC 1 ASC	5.0	13.1	BDL	1383	61.0	BDL	BDL
W2.11	H20 BC1 BSC	4.9	12.5	BDL	1346	61.5	BDL	BDL
W2.12	inflow cliffs	10.0	15.9	BDL	939	130.1	BDL	BDL
	average	**12.3**	**13.2**		**1724.8**	**78.5**		
	s.d.	**5**	**2**		**660**	**53**		









2. Unfiltered and 0.2 um filtered water

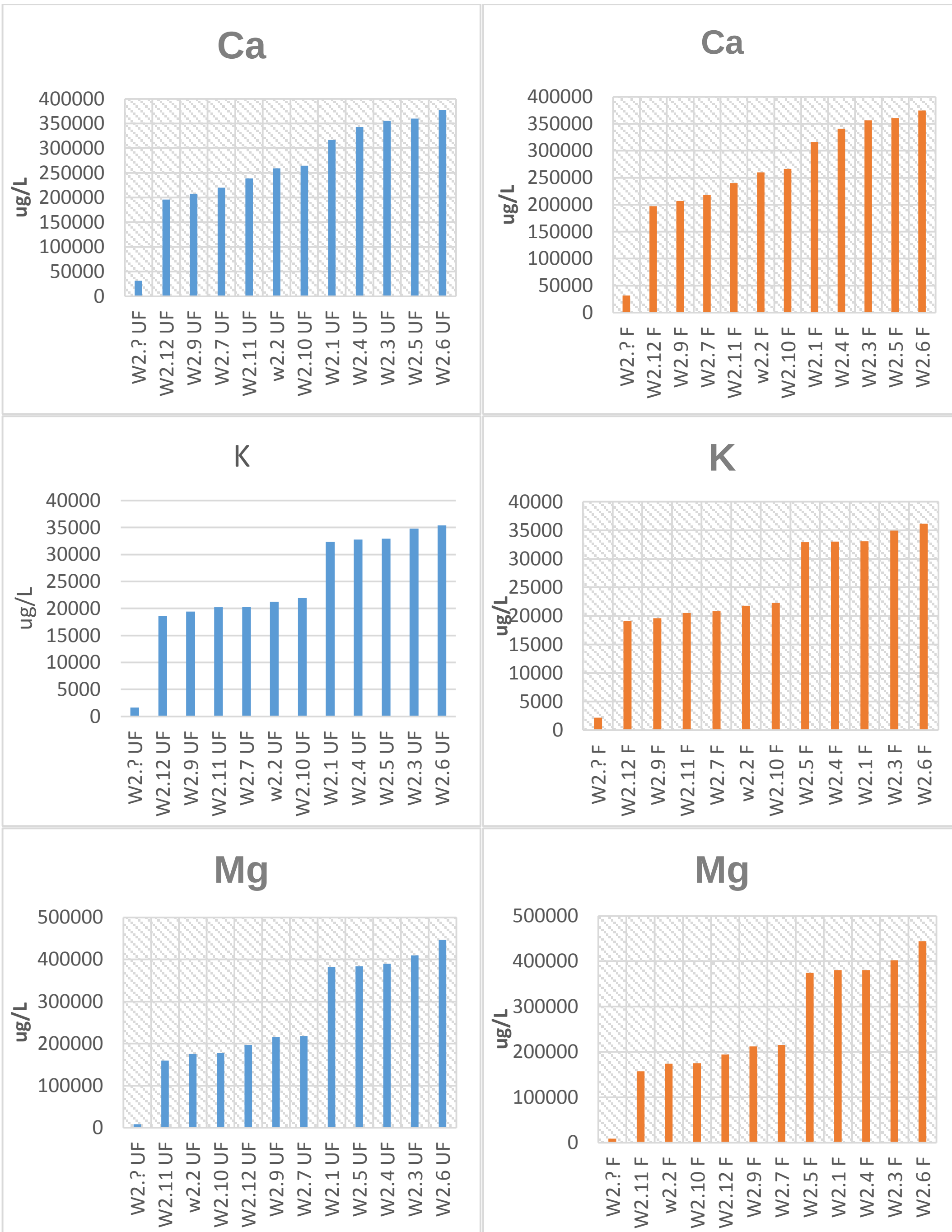
Unfiltered Total Recoverable						
Code	Location	**Na**	**Mg**	**K**	**Ca**	**Sr**
		ug/L	ug/L	ug/L	ug/L	ug/L
	M. DET. Lmt.	**0.8273**	**1.994**	**5.367**	**1.146**	**0.148**
W2.11 UF	H2O BC 1 BSC	7985	159800	20240	238800	2753
W2.10 UF	H2O BC 1 ASC	9972	177700	21940	264700	3032
W2.3 UF	BC# 2	15570	409900	34800	355500	3923
W2.5 UF	BC# 3	14200	383600	32940	359800	3856
W2.4 UF	BC# 2 Inflow	14730	389800	32770	343100	3807
W2.6 UF	BC# 3 Inflow	16400	447200	35390	376800	4912
w2.2 UF	BC 1 Outflow	10240	175400	21270	259200	3011
W2.1 UF	BC 2 Outflow	14740	381600	32330	316700	3453
W2.12 UF	Inflow Cliffs	9746	196500	18630	196000	532
W2.9 UF	Cliff pond Outflow	10540	215600	19410	207500	572
W2.7 UF	Cliff pond # 28	10950	218000	20310	220000	617
W2.? UF	Nist water	21350	8643	1665	31240	319
	Average	**12279**	**286827**	**26366**	**285282**	**2770**
	s.d	**2742**	**107976**	**6744**	**63773**	**1454**

	Filtered 0.2um	**Na**	**Mg**	**K**	**Ca**	**Sr**
W2.11 F	H2O BC 1 BSC	7921	157400	20510	240200	2755
W2.10 F	H2O BC 1 ASC	9613	175200	22270	266500	3059
W2.3 F	BC# 2	15520	402400	34930	356300	3910
W2.5 F	BC# 3	14080	375000	32940	360600	3866
W2.4 F	BC# 2 Inflow	14540	380800	33040	341000	3783
W2.6 F	BC# 3 Inflow	16210	444100	36190	374700	4954
w2.2 F	BC 1 Outflow	9947	173800	21790	260100	3059
W2.1 F	BC 2 Outflow	14640	380200	33060	316500	3425
W2.12 F	Inflow Cliffs	9675	194400	19140	197000	537
W2.9 F	Cliff pond Outflow	10380	212300	19620	206700	572
W2.7 F	Cliff pond # 28	10910	215300	20810	218300	624
W2.? F	Nist water	21330	8675	2156	31620	331

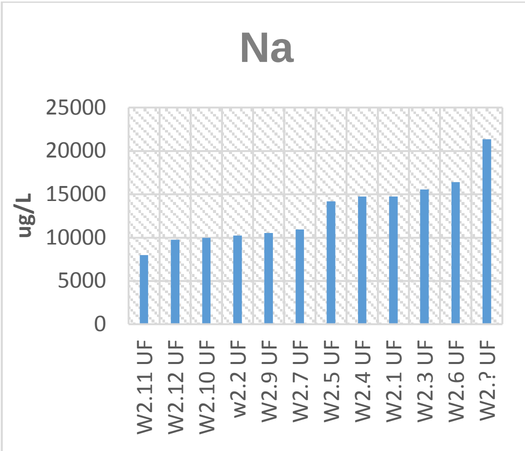
	Average	**12131**	**282809**	**26755**	**285264**	**2777**
	s.d	**2754**	**106361**	**6751**	**63436**	**1456**

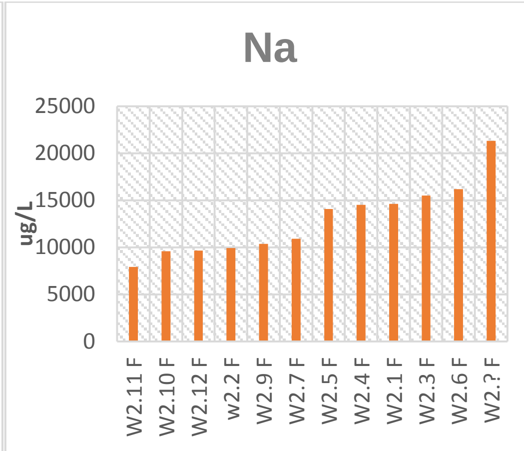
Method- ICP-MS Total recoverable by direct injection

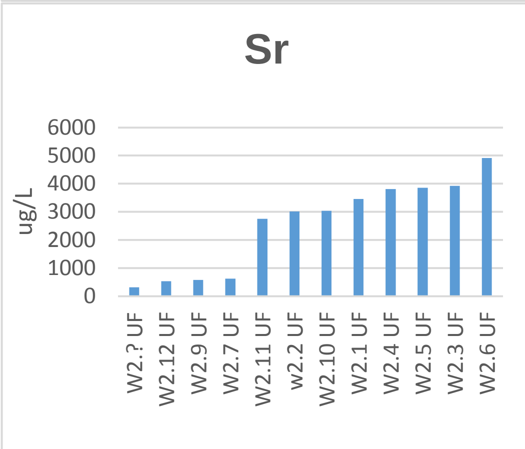
Unfiltered or filterd to 0.2 um

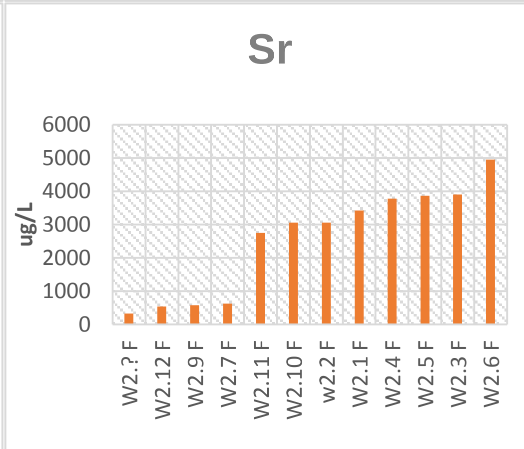


Blue: Filtered, Orange: Unfiltered









Blue: Filtered, Orange: Unfiltered

3. Conc. ug/Lof B, V, Cr, Mn , Fe, Co, Ni, Cu, Zn, As, Se

	ug/L	B	V	Cr	Mn	Fe	Co
w2.2	BC 1 Outflow	32.80	22.50	0.15	56.20	15.27	0.90
W2.1	BC 2 Outflow	70.65	38.35	**0.09**	17.80	6.98	0.40
W2.3	BC# 2	71.45	38.00	0.10	0.40	72.90	0.40
W2.4	BC# 2 Inflow	71.20	38.90	0.90	34.20	12.98	0.50
W2.5	BC# 3	70.65	36.30	0.10	48.20	18.92	0.50
W2.6	BC# 3 Inflow	74.25	44.15	**0.09**	2.20	**0.25**	0.40
W2.7	Cliff pond # 28	9.35	18.50	**0.09**	0.10	0.61	0.40

	ug/L	B	V	Cr	Mn	Fe	Co
W2.8	Cliff pond Outflow	9.50	19.70	0.10	0.20	8.64	0.40
W2.10	H2O BC 1 ASC	32.95	21.80	0.25	56.10	18.09	1.00
W2.11	H2O BC 1 BSC	31.00	20.90	0.25	1.00	15.67	0.40
W2.12	Inflow Cliffs	10.55	18.70	0.10	1.70	10.44	0.40
w2.2 F	BC 1 Outflow - filtered	42.15	23.25	**0.09**	**0.01**	**0.25**	0.40
W2.1 F	BC 2 Outflow - filtered	68.70	39.15	**0.09**	0.30	**0.25**	0.30
W2.3 F	BC# 2 - filtered	68.45	38.90	**0.09**	0.10	1.35	0.40
W2.4 F	BC# 2 Inflow - filtered	68.80	39.90	**0.09**	0.30	**0.25**	0.40
W2.5 F	BC# 3 - filtered	66.90	37.00	**0.09**	**0.01**	**0.25**	0.40
W2.6 F	BC# 3 Inflow - filtered	78.70	50.90	**0.09**	0.10	**0.25**	0.50
W2.7 F	Cliff pond # 28 - filtered	12.30	19.30	**0.09**	**0.01**	**0.25**	0.30
W2.9 F	Cliff pond Outflow - filtered	9.50	20.40	**0.09**	0.30	**0.25**	0.30
W2.00 F	Dupe 1-Cliff pond # 28	9.60	18.75	**0.09**	0.10	0.58	0.40
W2.11 F	H2O BC 1 ASC - filtered	33.80	21.55	**0.09**	0.20	0.32	0.40
W2.10 F	H2O BC 1 BSC - filtered	30.60	21.80	**0.09**	**0.01**	**0.25**	0.40
W2.12 F	Inflow Cliffs - filtered	10.55	18.05	**0.09**	**0.01**	**0.25**	0.30
W2.? F	Nist water	159.35	22.90	20.75	35.60	119.36	27.30
Wcert	Certified Value	154.00	36.93	19.90	38.02	98.10	26.40
	MDL	1.05	0.09	0.09	0.01	0.25	0.09
	Average	**51.91**	**29.06**	**1.76**	**11.73**	**16.11**	**2.56**
	s.d	**40**	**10**	**5**	**19**	**31**	**7**

Method -ICP-MS analysis -total recoverable by direct injection

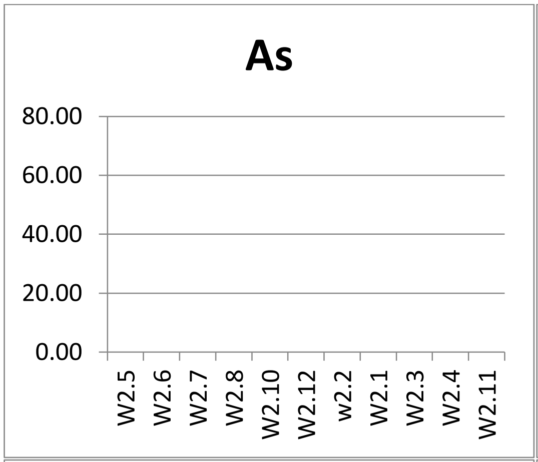
Unfiltered or filtered to 0.2 um

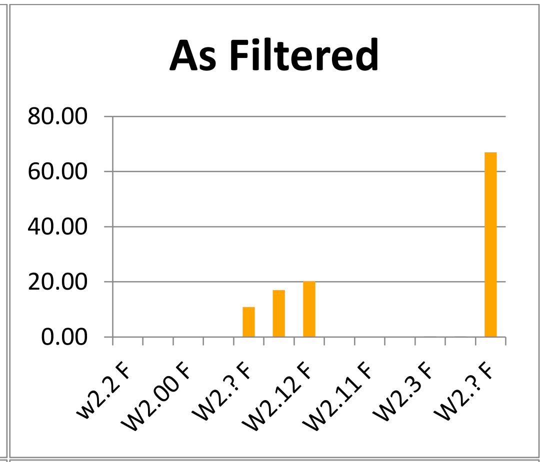
Nist Water and Certified values excluded from Average and Standard Deviation, yellow detection limit value

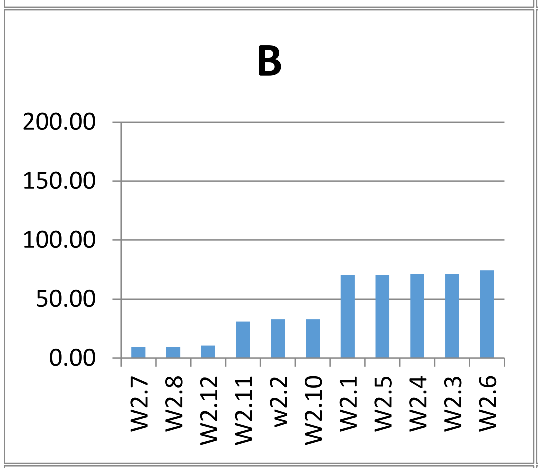
	ug/L	Ni	Cu	Zn	As	Se
w2.2	BC 1 Outflow	11.25	0.60	12.65	0.20	25.45
W2.1	BC 2 Outflow	2.20	0.10	5.15	0.20	10.20
W2.3	BC# 2	2.00	0.60	7.65	0.20	11.00
W2.4	BC# 2 Inflow	3.00	0.40	7.00	0.20	10.95
W2.5	BC# 3	3.05	0.40	7.90	0.15	11.40
W2.6	BC# 3 Inflow	4.15	0.30	6.55	0.15	17.10
W2.7	Cliff pond # 28	1.95	0.70	4.95	0.15	23.00
W2.8	Cliff pond Outflow	1.35	0.40	5.25	0.15	22.50
W2.10	H2O BC 1 ASC	11.80	0.70	18.20	0.15	26.75
W2.11	H2O BC 1 BSC	10.60	0.50	10.25	0.20	23.65
W2.12	Inflow Cliffs	2.00	0.40	6.05	0.15	22.20
w2.2 F	BC 1 Outflow - filtered	7.90	0.40	7.20	0.10	24.75
W2.1 F	BC 2 Outflow - filtered	1.75	0.60	5.70	0.15	9.50
W2.3 F	BC# 2 - filtered	1.95	0.20	8.20	0.20	10.20
W2.4 F	BC# 2 Inflow - filtered	2.10	0.20	5.60	0.20	10.05
W2.5 F	BC# 3 - filtered	2.70	0.30	6.90	0.15	10.30
W2.6 F	BC# 3 Inflow - filtered	3.10	1.70	8.25	0.15	17.05
W2.7 F	Cliff pond # 28 - filtered	1.75	0.30	4.55	0.10	21.95
W2.9 F	Cliff pond Outflow - filtered	1.45	0.50	5.40	0.15	21.75
W2.00 F	Dupe 1-Cliff pond # 28	1.80	0.10	5.20	0.10	21.40
W2.11 F	H2O BC 1 ASC - filtered	9.00	1.40	13.55	0.15	24.15
W2.10 F	H2O BC 1 BSC - filtered	8.80	0.40	8.95	0.15	24.10
W2.12 F	Inflow Cliffs - filtered	1.30	0.40	4.80	0.10	20.30
W2.? F	Nist water	71.10	28.00	96.50	66.95	10.90
Wcert	Certified Value	60.89	26.40	76.50	58.98	11.68
	MDL	0.09	0.01	0.23	0.09	0.23
	Average	**9.16**	**2.64**	**13.96**	**5.18**	**17.69**

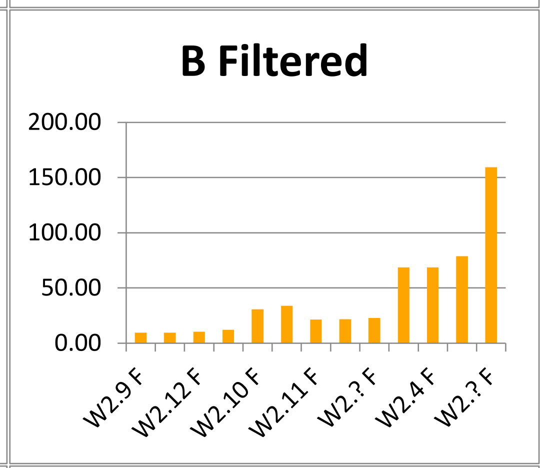
	ug/L	Ni	Cu	Zn	As	Se
	s.d	17	7	22	17	6

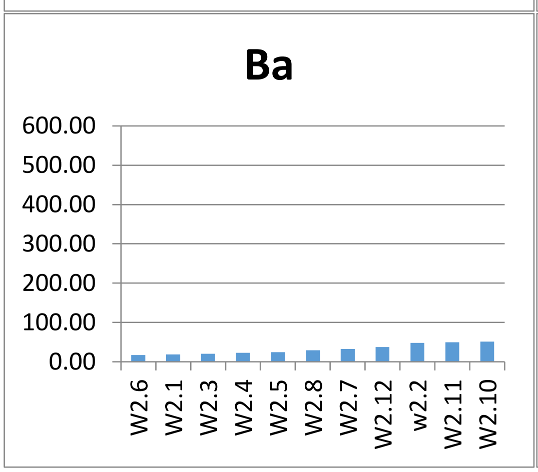
	ug/L	Sr	Mo	Cd	Ba	Pb
w2.2	BC 1 Outflow	3006.50	2.89	0.31	48.00	0.09
W2.1	BC 2 Outflow	3453.50	2.89	0.40	19.25	0.09
W2.3	BC# 2	3762.00	2.89	0.31	20.75	0.09
W2.4	BC# 2 Inflow	3797.50	2.89	0.31	22.85	0.09
W2.5	BC# 3	3883.00	2.89	0.31	24.70	0.09
W2.6	BC# 3 Inflow	4877.50	2.89	0.50	17.65	0.09
W2.7	Cliff pond # 28	543.00	2.89	0.31	32.55	0.09
W2.8	Cliff pond Outflow	527.00	2.89	0.31	29.80	0.09
W2.10	H2O BC 1 ASC	3100.00	2.89	0.31	51.20	0.09
W2.11	H2O BC 1 BSC	2861.50	2.89	0.31	49.90	0.09
W2.12	Inflow Cliffs	547.00	2.89	0.31	37.75	0.09
w2.2 F	BC 1 Outflow - filtered	2946.50	2.89	0.31	46.70	0.09
W2.1 F	BC 2 Outflow - filtered	2606.00	2.89	0.31	11.70	0.09
W2.3 F	BC# 2 - filtered	3535.00	2.89	0.31	20.00	0.09
W2.4 F	BC# 2 Inflow - filtered	3594.00	2.89	0.31	18.25	0.09
W2.5 F	BC# 3 - filtered	3639.50	2.89	0.31	22.95	0.09
W2.6 F	BC# 3 Inflow - filtered	4473.50	2.89	0.31	13.05	0.09
W2.7 F	Cliff pond # 28 - filtered	536.00	2.89	0.40	33.20	0.09
W2.9 F	Cliff pond Outflow - filtered	522.00	2.89	0.31	31.05	0.09
W2.00 F	Dupe 1-Cliff pond # 28	532.50	2.89	0.31	32.20	0.09
W2.11 F	H2O BC 1 ASC - filtered	2845.50	2.89	0.31	47.00	0.09
W2.10 F	H2O BC 1 BSC - filtered	2792.00	2.89	0.31	48.85	0.09
W2.12 F	Inflow Cliffs - filtered	507.00	2.89	0.31	33.75	0.09
W2.? F	Nist water	322.00	79.50	6.80	547.50	16.55
Wcert	Certified Value	315.20	118.50	6.41	531.00	19.15
	MDL	0.43	2.89	0.31	0.09	0.09
	Average	**2381.01**	**10.58**	**0.83**	**71.66**	**1.51**
	s.d	**1506**	**27**	**2**	**138**	**5**

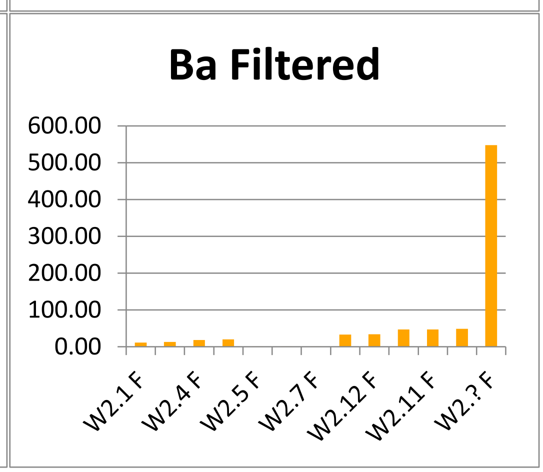




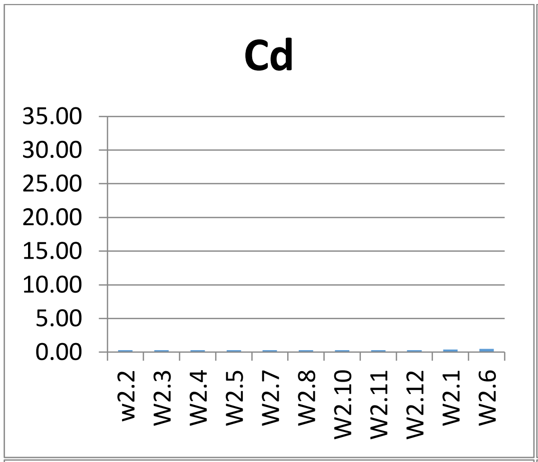


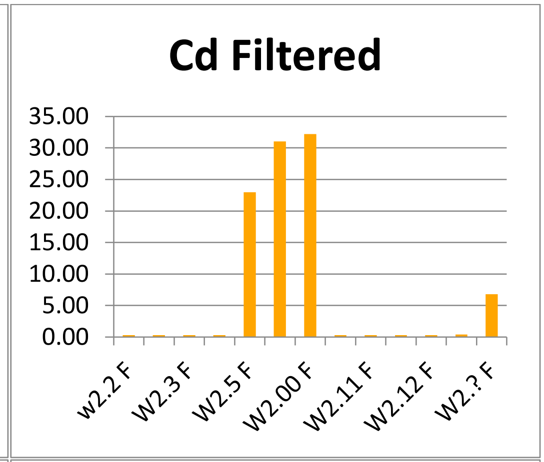


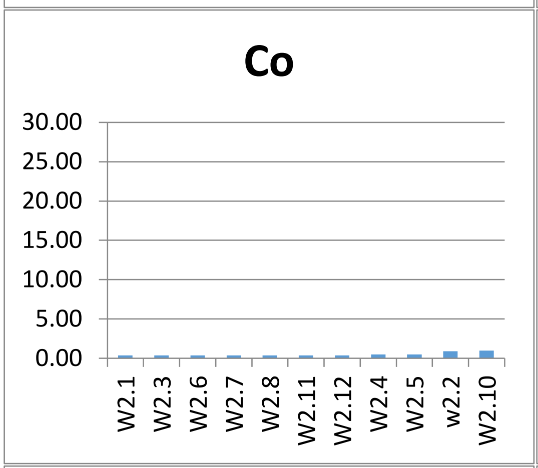


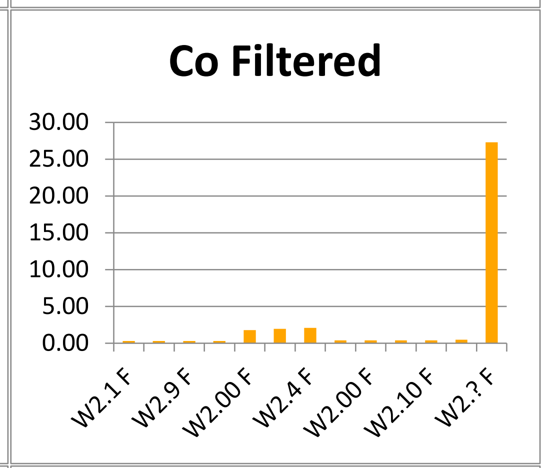


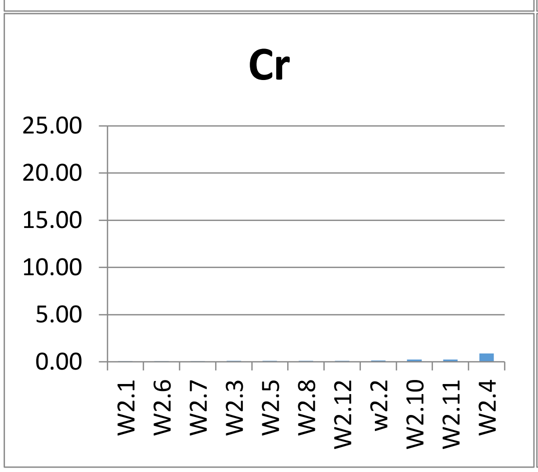
All Charts are in ug/L

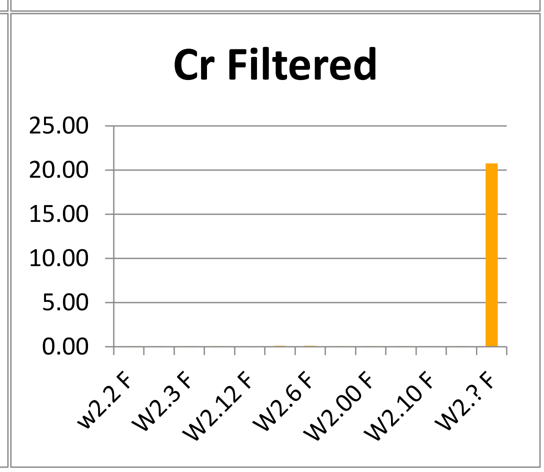

Cd
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
w2.2
W2.3
W2.4
W2.5
W2.7
W2.8
W2.10
W2.11
W2.12
W2.1
W2.6

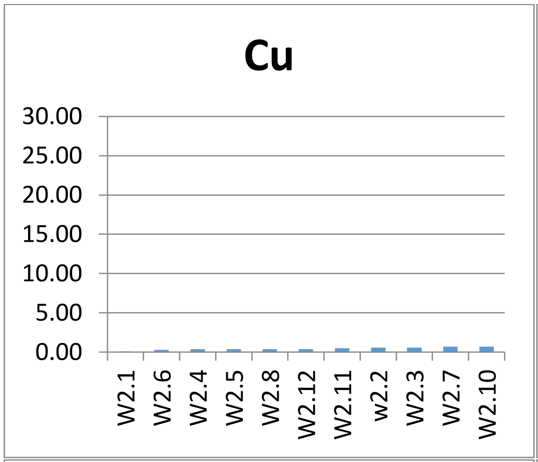

Cd Filtered
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
w2.2 F
W2.3 F
W2.5 F
W2.00 F
W2.11 F
W2.12 F
W2.? F

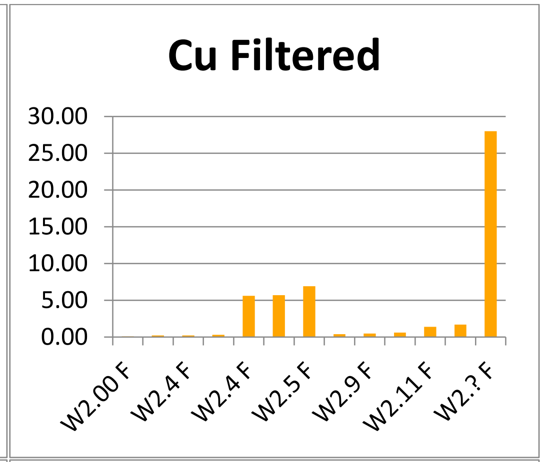

Co
30.00
25.00
20.00
15.00
10.00
5.00
0.00
W2.1
W2.3
W2.6
W2.7
W2.8
W2.11
W2.12
W2.4
W2.5
w2.2
W2.10

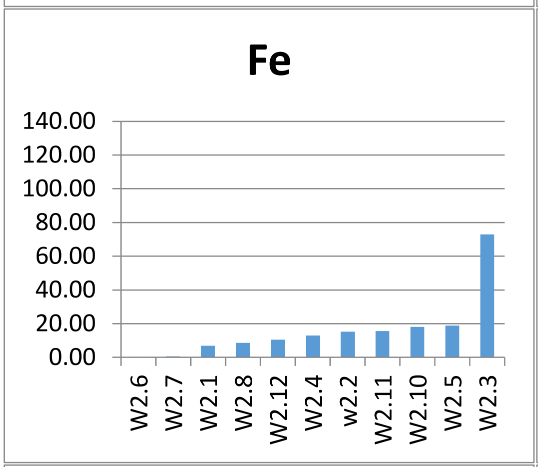

Co Filtered
30.00
25.00
20.00
15.00
10.00
5.00
0.00
W2.1 F
W2.9 F
W2.00 F
W2.4 F
W2.00 F
W2.10 F
W2.? F

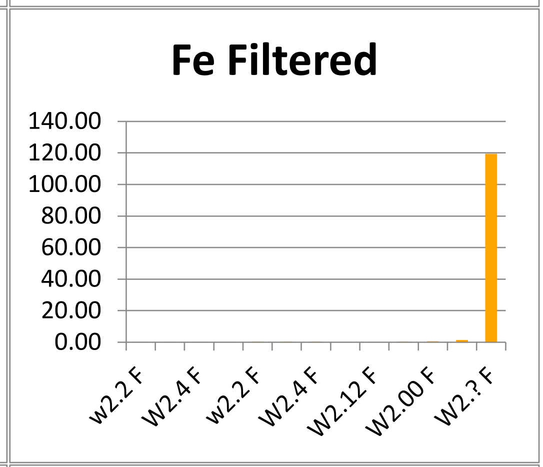

Cr
25.00
20.00
15.00
10.00
5.00
0.00
W2.1
W2.6
W2.7
W2.3
W2.5
W2.8
W2.12
w2.2
W2.10
W2.11
W2.4

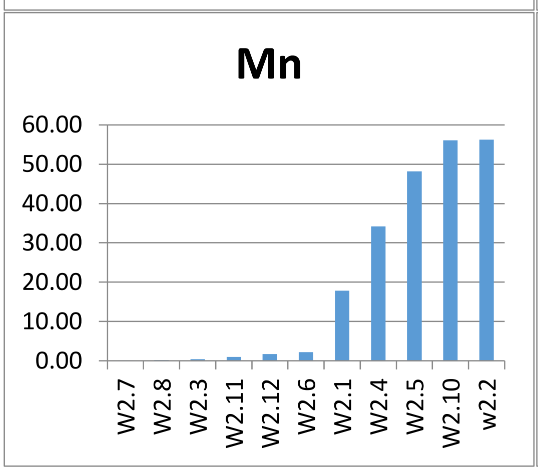

Cr Filtered
25.00
20.00
15.00
10.00
5.00
0.00
w2.2 F
W2.3 F
W2.12 F
W2.6 F
W2.00 F
W2.10 F
W2.? F

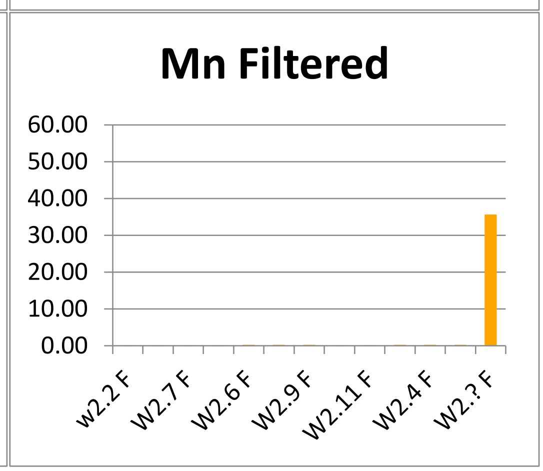

Cu
30.00
25.00
20.00
15.00
10.00
5.00
0.00
W2.1
W2.6
W2.4
W2.5
W2.8
W2.12
W2.11
w2.2
W2.3
W2.7
W2.10

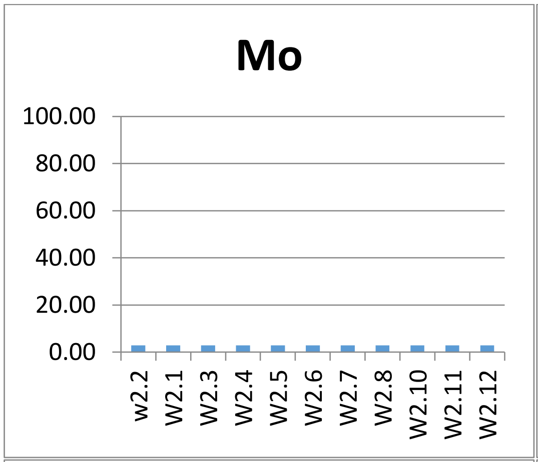

Cu Filtered
30.00
25.00
20.00
15.00
10.00
5.00
0.00
W2.00 F
W2.4 F
W2.4 F
W2.5 F
W2.9 F
W2.11 F
W2.? F

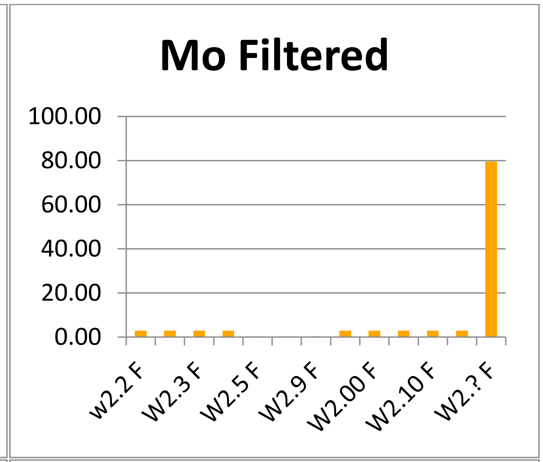

Fe
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
W2.6
W2.7
W2.1
W2.8
W2.12
W2.4
w2.2
W2.11
W2.10
W2.5
W2.3

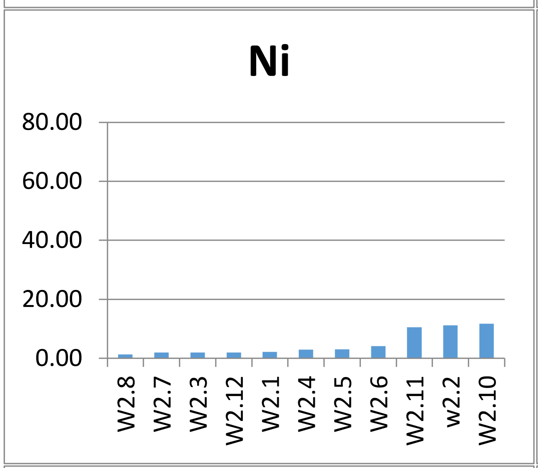

Fe Filtered
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
w2.2 F
W2.4 F
w2.2 F
W2.4 F
W2.12 F
W2.00 F
W2.? F

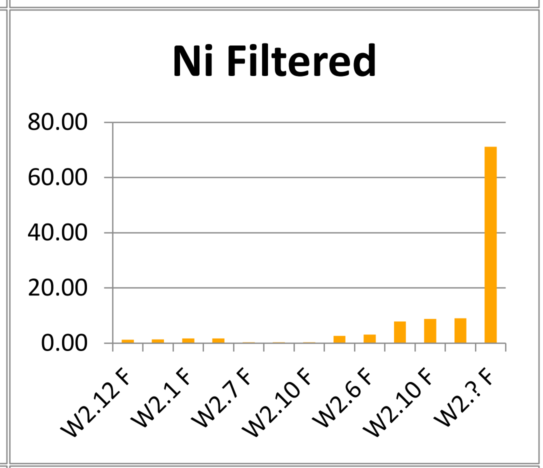

Mn
60.00
50.00
40.00
30.00
20.00
10.00
0.00
W2.7
W2.8
W2.3
W2.11
W2.12
W2.6
W2.1
W2.4
W2.5
W2.10
w2.2

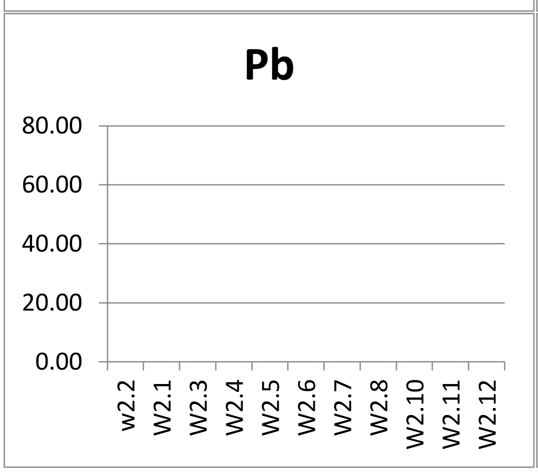

Mn Filtered
60.00
50.00
40.00
30.00
20.00
10.00
0.00
w2.2 F
W2.7 F
W2.6 F
W2.9 F
W2.11 F
W2.4 F
W2.? F

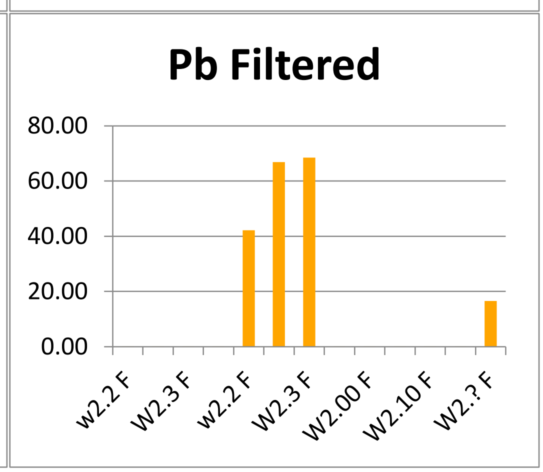

Mo
100.00
80.00
60.00
40.00
20.00
0.00
w2.2
W2.1
W2.3
W2.4
W2.5
W2.6
W2.7
W2.8
W2.10
W2.11
W2.12

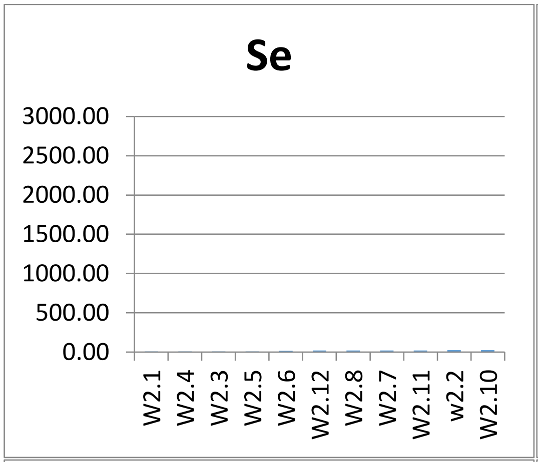

Mo Filtered
100.00
80.00
60.00
40.00
20.00
0.00
w2.2 F
W2.3 F
W2.5 F
W2.9 F
W2.00 F
W2.10 F
W2.? F

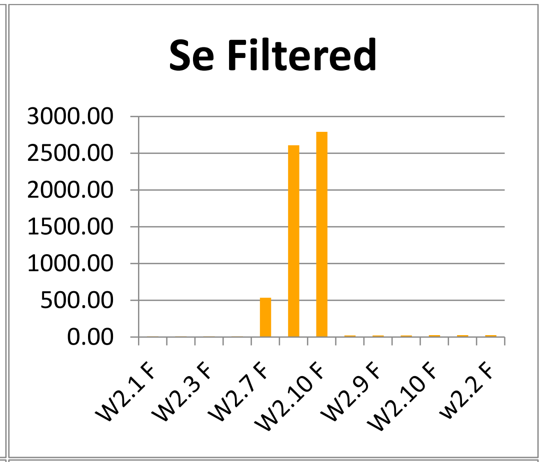

Ni
80.00
60.00
40.00
20.00
0.00
W2.8
W2.7
W2.3
W2.12
W2.1
W2.4
W2.5
W2.6
W2.11
w2.2
W2.10

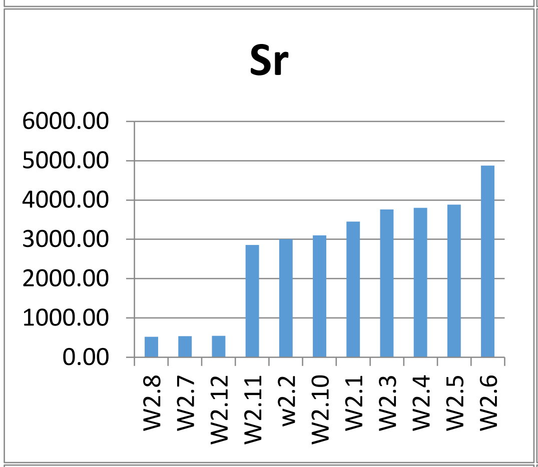

Ni Filtered
80.00
60.00
40.00
20.00
0.00
W2.12 F
W2.1 F
W2.7 F
W2.10 F
W2.6 F
W2.10 F
W2.? F

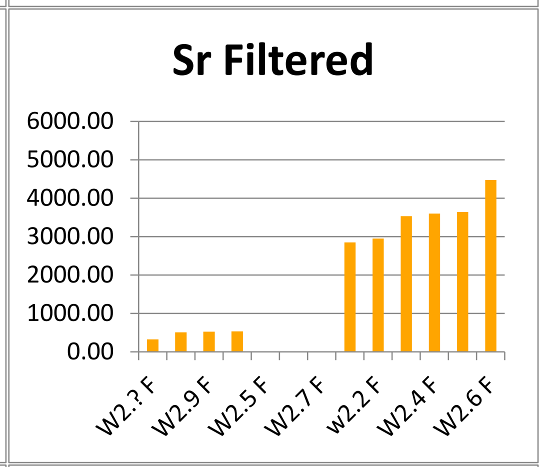

Pb
80.00
60.00
40.00
20.00
0.00
w2.2
W2.1
W2.3
W2.4
W2.5
W2.6
W2.7
W2.8
W2.10
W2.11
W2.12

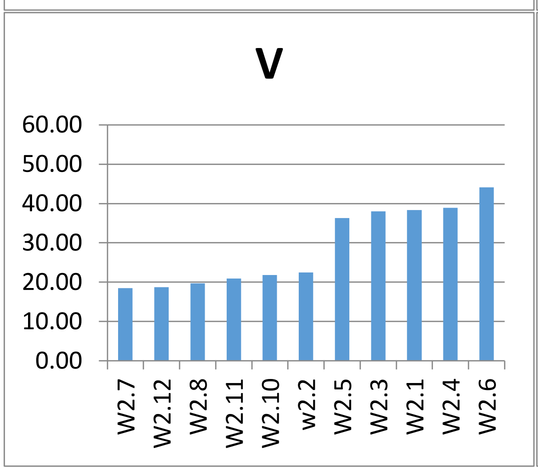

Pb Filtered
80.00
60.00
40.00
20.00
0.00
w2.2 F
W2.3 F
w2.2 F
W2.3 F
W2.00 F
W2.10 F
W2.? F

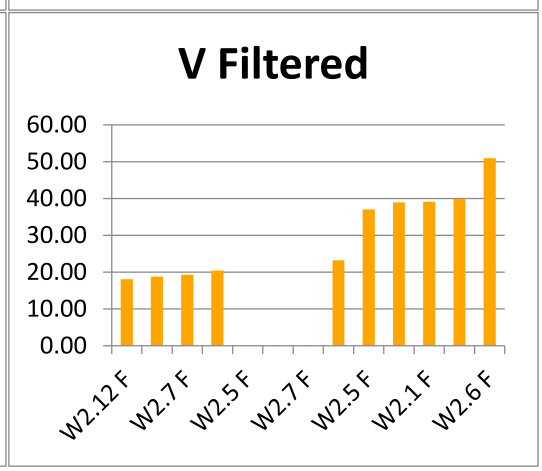

Se
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
W2.1
W2.4
W2.3
W2.5
W2.6
W2.12
W2.8
W2.7
W2.11
w2.2
W2.10

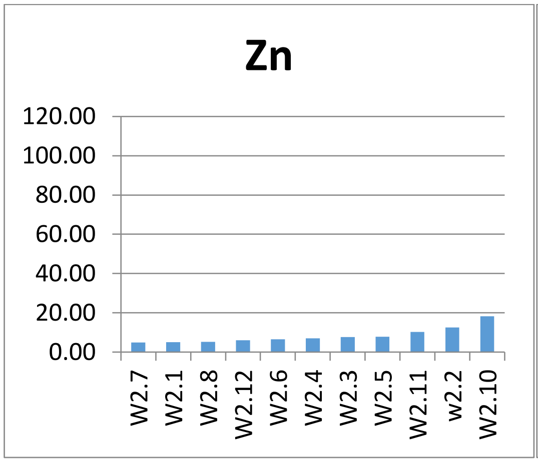

Se Filtered
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
W2.1 F
W2.3 F
W2.7 F
W2.10 F
W2.9 F
W2.10 F
w2.2 F

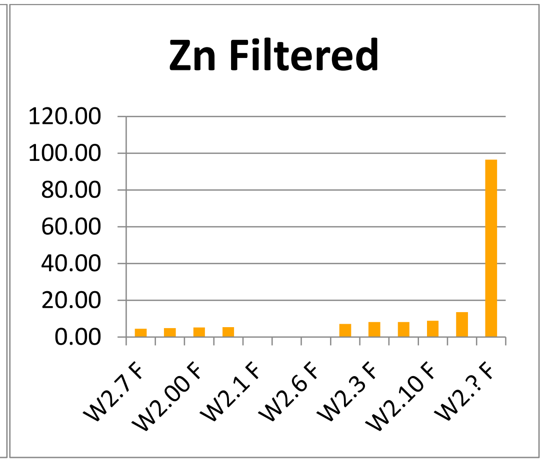

Sr
6000.00
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
W2.8
W2.7
W2.12
W2.11
w2.2
W2.10
W2.1
W2.3
W2.4
W2.5
W2.6


Sr Filtered
6000.00
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
W2.? F
W2.9 F
W2.5 F
W2.7 F
w2.2 F
W2.4 F
W2.6 F


V
60.00
50.00
40.00
30.00
20.00
10.00
0.00
W2.7
W2.12
W2.8
W2.11
W2.10
w2.2
W2.5
W2.3
W2.1
W2.4
W2.6


V Filtered
60.00
50.00
40.00
30.00
20.00
10.00
0.00
W2.12 F
W2.7 F
W2.5 F
W2.7 F
W2.5 F
W2.1 F
W2.6 F





4. Sediment cores profiles all elements Cliff's and BC 1

	Sample ID	cm from surface	Core	B	V	Cr	Mn	Co
MK-001	Cliff Pond Core - 2-4	8-10	Cliff pond	**0.27**	10.59	10.39	491.4	10.04
MK-002	Cliff Pond Core - 4-6	6-8	Cliff pond	**0.27**	15.33	13.37	292.6	8.29
MK-003	Cliff Pond Core - 6-8	4-6	Cliff pond	**0.27**	15.74	14.41	229.7	11.50
MK-004	Cliff Pond Core - 8-10	2-4	Cliff pond	**0.27**	13.69	12.76	301.1	10.13
MK-005	Cliff Pond Core - 10-12	0-2	Cliff pond	**0.27**	13.57	11.71	769.1	8.77
MK-006	BC #1- 0-2	22-24	BC#1	**0.27**	13.77	11.95	289.5	10.41
MK-007	BC #1- 2-4	20-22	BC#1	**0.27**	17.48	16.44	283.4	10.30
MK-008	BC #1- 4-6	18-20	BC#1	**0.27**	15.56	14.56	326.6	10.48
MK-009	BC #1- 6-8	16-18	BC#1	**0.27**	16.05	16.37	336.0	12.25
MK-010	BC #1- 8-10	14-16	BC#1	**0.27**	12.06	13.30	356.0	13.40
MK-011	BC #1- 10-12	12-14	BC#1	1.71	15.29	13.84	340.6	13.35
MK-012	BC #1- 12-14	10-12	BC#1	0.60	13.46	12.69	350.7	13.60
MK-013	BC #1- 14-16	8-10	BC#1	1.68	14.76	13.20	299.2	11.64
MK-014	BC #1- 16-18	6-8	BC#1	0.58	13.59	11.64	344.7	12.88
MK-015	BC #1- 18-20	4-6	BC#1	1.71	14.88	13.13	343.6	12.40
MK-016	BC #1- 20-22	2-4	BC#1	0.68	13.62	12.73	365.3	13.60
MK-017	BC #1- 22-24	0-2	BC#1	**0.27**	11.55	11.33	374.1	13.96
MK-018	Dupe 1- Cliff Pond Core -6-8	6-8	Cliff pond	**0.27**	9.85	6.82	318.4	9.25
MK-019	Dupe 2- Cliff Pond Core -2-4	2-4	Cliff pond	**0.27**	11.98	10.26	337.7	11.55
MK-020	Dupe 3-BC #1- 2-4	20-22	BC#1	**0.27**	13.24	12.18	308.8	11.27
MK-021	Dupe 4-BC #1- 18-20	4-6	BC#1	1.28	13.50	12.51	354.5	12.87
MK-022	SRM1- Montana II			4.05	23.37	12.04	326.8	4.89
MK-023	SRM2- Montana II			3.52	21.81	12.56	326.8	4.87
	Average			**0.85**	**14.55**	**12.62**	**350.72**	**10.94**
	Sd			**1**	**3**	**2**	**100**	**2**
	Certified acid leachable				28	15	460	7.5
	Method detection limit			**0.27**	**0.02**	**0.07**	**0.03**	**0.03**

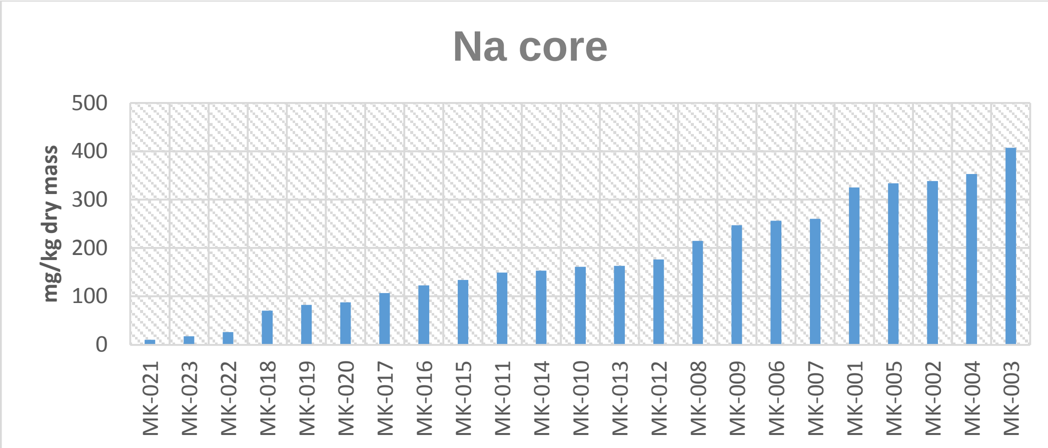
MK-001	Cliff Pond Core - 2-4	8-10	Cliff pond	**Ni**	**Cu**	**Zn**	**As**	**Se**
MK-002	Cliff Pond Core - 4-6	6-8	Cliff pond	25.29	20.29	67.49	3.62	0.75
MK-003	Cliff Pond Core - 6-8	4-6	Cliff pond	27.43	23.67	68.44	4.24	0.60
MK-004	Cliff Pond Core - 8-10	2-4	Cliff pond	36.68	25.50	77.68	4.06	1.37
MK-005	Cliff Pond Core - 10-12	0-2	Cliff pond	35.68	24.20	85.93	4.00	7.44
MK-006	BC #1- 0-2	22-24	BC#1	39.17	19.68	109.58	3.28	17.01
MK-007	BC #1- 2-4	20-22	BC#1	28.81	22.76	78.10	4.42	0.66
MK-008	BC #1- 4-6	18-20	BC#1	35.66	24.60	91.23	4.84	1.26
MK-009	BC #1- 6-8	16-18	BC#1	34.76	25.52	94.35	4.84	1.49
MK-010	BC #1- 8-10	14-16	BC#1	38.26	26.14	94.28	5.16	0.96
MK-011	BC #1- 10-12	12-14	BC#1	36.31	26.56	93.61	5.45	0.74
MK-012	BC #1- 12-14	10-12	BC#1	40.45	28.42	99.74	5.74	0.92
MK-013	BC #1- 14-16	8-10	BC#1	38.62	27.09	95.86	5.42	0.90
MK-014	BC #1- 16-18	6-8	BC#1	34.21	24.03	87.26	5.11	0.96
MK-015	BC #1- 18-20	4-6	BC#1	36.36	26.88	96.54	5.80	1.15
MK-016	BC #1- 20-22	2-4	BC#1	34.51	24.82	88.82	5.78	0.90
MK-017	BC #1- 22-24	0-2	BC#1	36.96	26.55	93.74	5.78	0.82
MK-018	Dupe 1- Cliff Pond Core -6-8	6-8	Cliff pond	35.93	25.74	91.78	5.50	0.72
MK-019	Dupe 2- Cliff Pond Core -2-4	2-4	Cliff pond	25.31	23.35	67.39	3.76	0.53
MK-020	Dupe 3-BC #1- 2-4	20-22	BC#1	38.71	26.43	95.80	4.36	7.44
MK-021	Dupe 4-BC #1- 18-20	4-6	BC#1	35.75	25.74	94.36	4.77	1.39
MK-022	SRM1- Montana II			34.75	25.05	90.56	5.63	0.84
MK-023	SRM2- Montana II			18.24	133.77	400.04	103.65	1.80
	Average			17.59	134.75	394.83	102.52	1.81
	Sd			**33.28**	**34.41**	**115.54**	**13.38**	**2.28**
				6	**31**	**88**	**28**	**4**
	Certified acid leachable							
	Method detection limit			15	130	350	89	1.7
				0.12	**1.07**	**2.83**	**0.03**	**0.03**

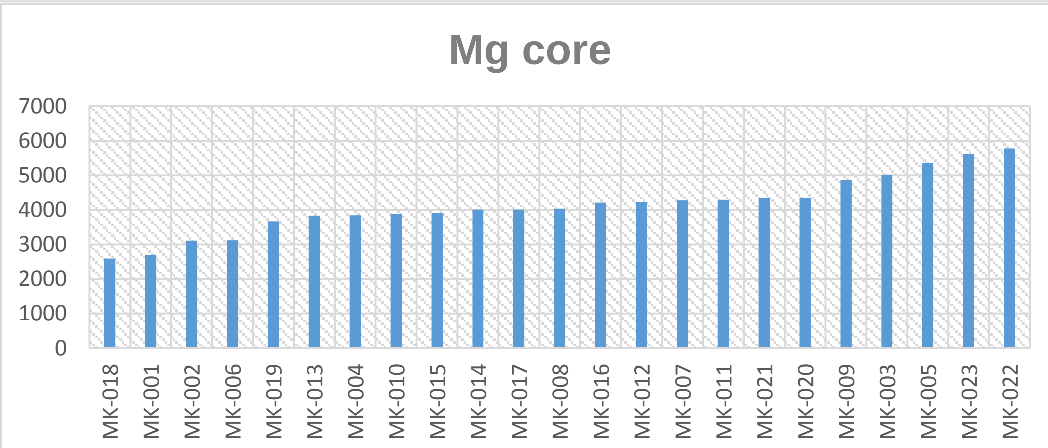
				Sr	Mo	Cd	Ba	Pb
MK-001	Cliff Pond Core - 2-4	8-10	Cliff pond	6.48	**0.72**	**0.02**	70.54	14.39
MK-002	Cliff Pond Core - 4-6	6-8	Cliff pond	9.75	**0.72**	**0.02**	114.92	16.93
MK-003	Cliff Pond Core - 6-8	4-6	Cliff pond	10.58	**0.72**	0.04	149.91	19.39
MK-004	Cliff Pond Core - 8-10	2-4	Cliff pond	18.59	**0.72**	0.02	116.10	17.65
MK-005	Cliff Pond Core - 10-12	0-2	Cliff pond	94.92	**0.72**	0.14	112.17	14.07
MK-006	BC #1- 0-2	22-24	BC#1	12.93	**0.72**	**0.02**	121.82	16.56
MK-007	BC #1- 2-4	20-22	BC#1	13.41	**0.72**	**0.02**	116.17	18.64
MK-008	BC #1- 4-6	18-20	BC#1	18.85	**0.72**	**0.02**	111.87	18.12
MK-009	BC #1- 6-8	16-18	BC#1	16.51	**0.72**	**0.02**	114.55	19.59
MK-010	BC #1- 8-10	14-16	BC#1	11.55	**0.72**	**0.02**	100.94	19.07
MK-011	BC #1- 10-12	12-14	BC#1	13.13	**0.72**	0.10	136.20	21.62
MK-012	BC #1- 12-14	10-12	BC#1	12.65	0.74	0.08	115.16	20.24
MK-013	BC #1- 14-16	8-10	BC#1	12.54	**0.72**	0.08	106.93	17.87
MK-014	BC #1- 16-18	6-8	BC#1	11.68	0.89	0.10	101.07	19.33
MK-015	BC #1- 18-20	4-6	BC#1	12.05	0.73	0.10	111.32	18.42
MK-016	BC #1- 20-22	2-4	BC#1	12.43	0.84	0.10	116.56	19.79
MK-017	BC #1- 22-24	0-2	BC#1	12.32	0.74	0.10	110.95	21.87
MK-018	Dupe 1- Cliff Pond Core -6-8	6-8	Cliff pond	5.62	**0.72**	0.08	86.99	16.02
MK-019	Dupe 2- Cliff Pond Core -2-4	2-4	Cliff pond	18.44	0.81	0.14	118.42	19.80
MK-020	Dupe 3-BC #1- 2-4	20-22	BC#1	12.28	0.83	0.12	97.48	18.66

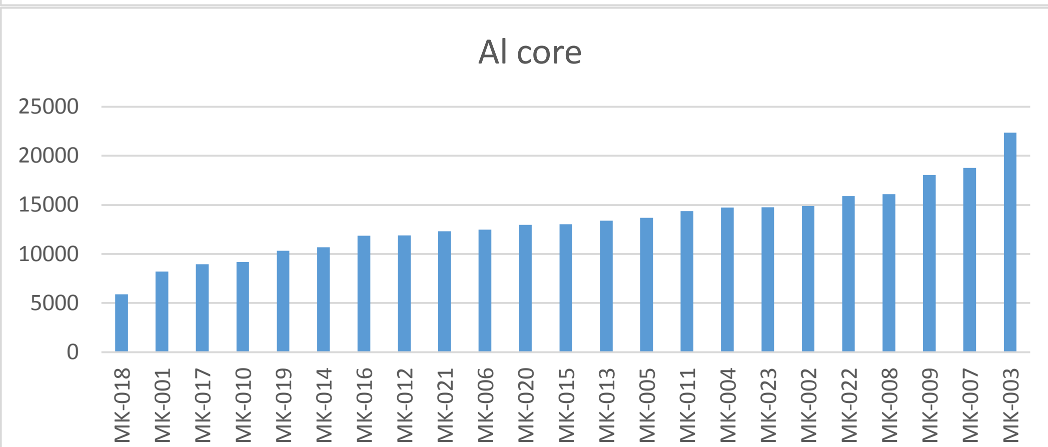
				Sr	Mo	Cd	Ba	Pb
MK-021	Dupe 4-BC #1- 18-20	4-6	BC#1	11.63	1.08	0.08	102.77	18.09
MK-022	SRM1- Montana II			13.42	1.11	44.64	181.39	1048.35
MK-023	SRM2- Montana II			13.26	1.09	44.77	178.91	1038.77
	Average			**16.30**	**0.79**	**3.95**	**117.09**	**107.53**
	Sd			**17.05**	**0.13**	**12.58**	**24.54**	**288.87**
	Certified acid leachable					47	190	1300
	Method detection limit			**0.06**	**0.72**	**0.02**	**0.03**	**0.16**

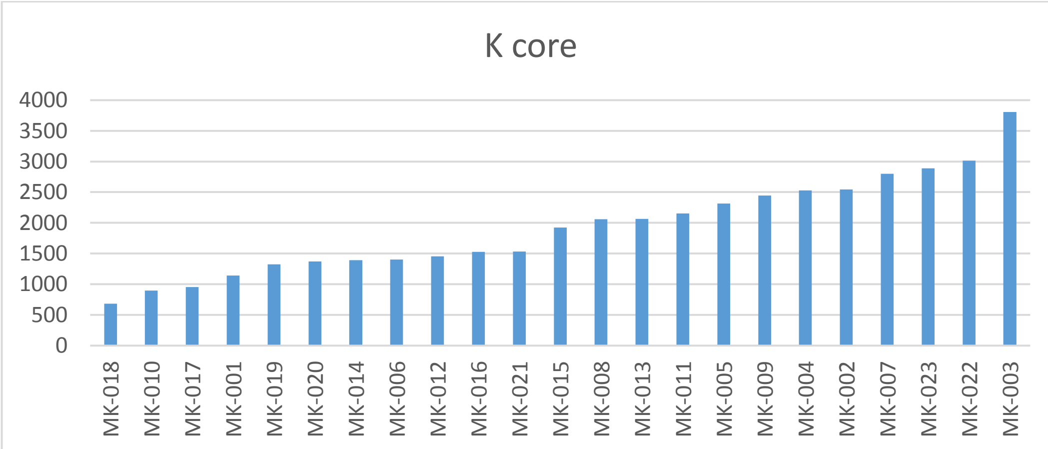
5. Sediment profiles all elements continued

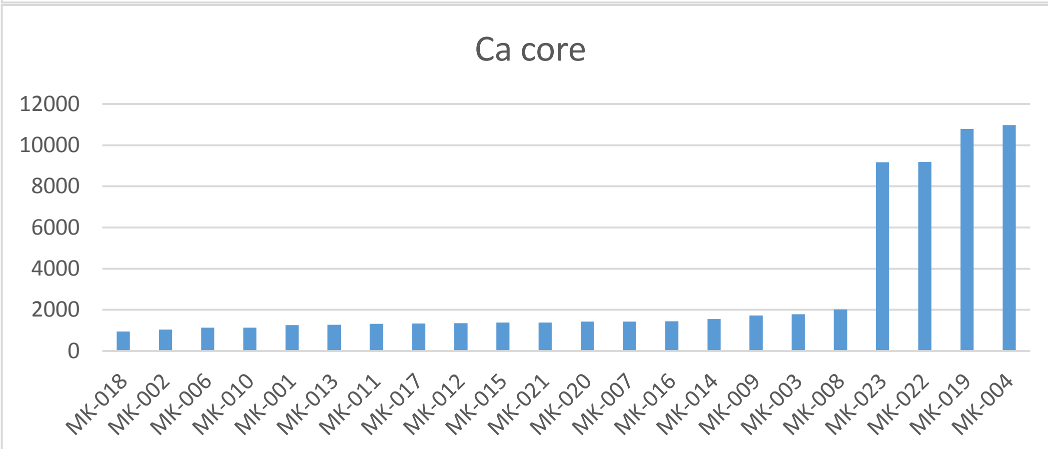
		mg/kg dry mass					
		Na	Mg	Al	K	Ca	Fe
MK-001	Cliff Pond Core - 2-4	325.53	2696.32	8189.33	1141.31	1262.55	21649.28
MK-002	Cliff Pond Core - 4-6	338.50	3108.59	14898.57	2545.74	1039.98	22712.81
MK-003	Cliff Pond Core - 6-8	407.05	5006.97	22355.05	3805.54	1788.20	32895.00
MK-004	Cliff Pond Core - 8-10	353.03	3840.80	14742.29	2529.35	10973.13	23820.90
MK-005	Cliff Pond Core - 10-12	333.66	5347.69	13668.51	2317.27	66357.17	17856.58
MK-006	BC #1- 0-2	256.74	3120.88	12485.51	1400.60	1135.26	21958.04
MK-007	BC #1- 2-4	260.51	4273.47	18772.17	2800.48	1431.13	25313.93
MK-008	BC #1- 4-6	214.88	4031.75	16113.10	2059.52	2021.83	24861.11
MK-009	BC #1- 6-8	246.95	4872.92	18044.83	2445.47	1729.64	26956.17
MK-010	BC #1- 8-10	161.26	3874.18	9171.81	894.68	1139.16	20864.03
MK-011	BC #1- 10-12	148.98	4296.38	14378.93	2154.09	1325.67	26729.56
MK-012	BC #1- 12-14	176.06	4216.29	11884.09	1453.10	1358.30	25512.85
MK-013	BC #1- 14-16	162.97	3826.64	13399.24	2063.11	1283.60	22848.01
MK-014	BC #1- 16-18	152.99	4007.55	10691.44	1388.44	1562.29	26008.34
MK-015	BC #1- 18-20	133.84	3917.06	13044.42	1924.72	1379.52	23211.48
MK-016	BC #1- 20-22	122.88	4211.05	11853.18	1525.63	1448.04	25912.63
MK-017	BC #1- 22-24	107.08	4007.98	8941.18	953.34	1341.77	23449.65
MK-018	Dupe 1- Cliff Pond Core -6-8	70.40	2590.47	5894.80	682.82	954.91	20565.55
MK-019	Dupe 2- Cliff Pond Core -2-4	82.71	3661.67	10323.73	1323.13	10791.55	23529.41
MK-020	Dupe 3-BC #1- 2-4	87.44	4348.17	12955.09	1370.63	1423.89	24582.67
MK-021	Dupe 4-BC #1- 18-20	**10.00**	4344.70	12317.97	1530.42	1379.61	23259.53
MK-022	SRM1- Montana II	26.45	5780.54	15895.01	3015.59	9184.92	17448.19
MK-023	SRM2- Montana II	17.50	5618.29	14767.40	2888.67	9165.01	16763.42
	Average	**182.50**	**4130.45**	**13251.64**	**1922.33**	**5716.40**	**23422.14**
	s.d	**115.07**	**823.84**	**3666.27**	**786.79**	**13639.59**	**3499.93**
	Acid Leachable	140-210	5000-6600	9800-15000	3300-4600	14000-17001	14000-18002
	MDL	**10**	**7.89**	**26.32**	**221.14**	**20.58**	**37.82**

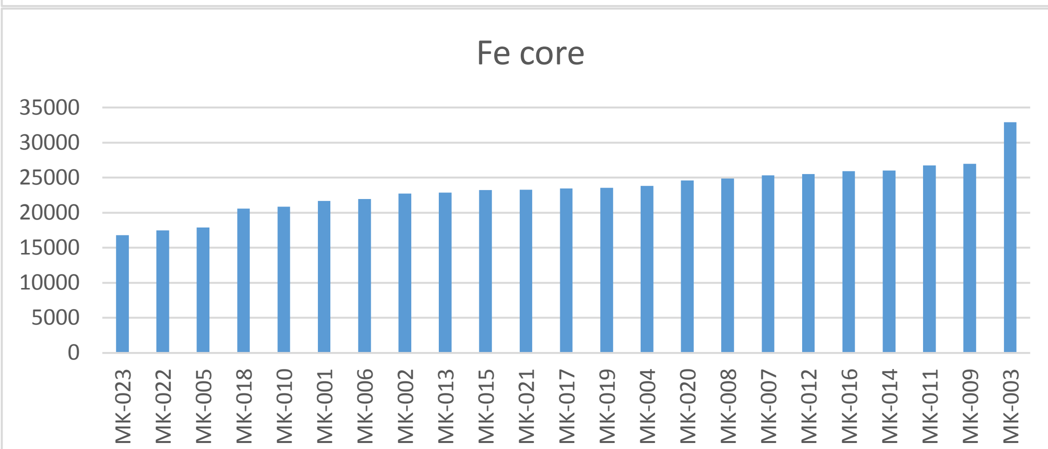

Na core
mg/kg dry mass
500
400
300
200
100
0
MK-021
MK-023
MK-022
MK-018
MK-019
MK-020
MK-017
MK-016
MK-015
MK-011
MK-014
MK-010
MK-013
MK-012
MK-008
MK-009
MK-006
MK-007
MK-001
MK-005
MK-002
MK-004
MK-003


Mg core
7000
6000
5000
4000
3000
2000
1000
0
MK-018
MK-001
MK-002
MK-006
MK-019
MK-013
MK-004
MK-010
MK-015
MK-014
MK-017
MK-008
MK-016
MK-012
MK-007
MK-011
MK-021
MK-020
MK-009
MK-003
MK-005
MK-023
MK-022


Al core
25000
20000
15000
10000
5000
0
MK-018
MK-001
MK-017
MK-010
MK-019
MK-014
MK-016
MK-012
MK-021
MK-006
MK-020
MK-015
MK-013
MK-005
MK-011
MK-004
MK-023
MK-002
MK-022
MK-008
MK-009
MK-007
MK-003


K core
4000
3500
3000
2500
2000
1500
1000
500
0
MK-018
MK-010
MK-017
MK-001
MK-019
MK-020
MK-014
MK-006
MK-012
MK-016
MK-021
MK-015
MK-008
MK-013
MK-011
MK-005
MK-009
MK-004
MK-002
MK-007
MK-023
MK-022
MK-003


Ca core
12000
10000
8000
6000
4000
2000
0
MK-018
MK-002
MK-006
MK-010
MK-001
MK-013
MK-011
MK-017
MK-012
MK-015
MK-021
MK-020
MK-007
MK-016
MK-014
MK-009
MK-003
MK-008
MK-023
MK-022
MK-019
MK-004


Fe core
35000
30000
25000
20000
15000
10000
5000
0
MK-023
MK-022
MK-005
MK-018
MK-010
MK-001
MK-006
MK-002
MK-013
MK-015
MK-021
MK-017
MK-019
MK-004
MK-020
MK-008
MK-007
MK-012
MK-016
MK-014
MK-011
MK-009
MK-003

6. Vegetation/ its parts, algae and debris all [elements]

		mg/kg dry mass				
	Sample	**B**	**Na**	**Mg**	**Al**	**K**
P1	BC #1	3.85	85	4972	13736	2771
P2	BC #1 Pond weed	11.00	994	3779	1279	24985
P3	BC #1 Pond weed Wash	BDL	352	8107	19596	6197
P4	BC #1 Before S. Curtain	4.02	97	5399	13907	2760
P5	BC #1 Leaf Debris	49.58	145	4721	2138	2328
P6	BC #1 Leaf Debris Wash	BDL	273	9362	22440	4301
P7	BC #1 BSC Floating cattail Debris	13.69	202	5105	6457	2092
P8	BC #1 BSC Floating cattail Debris Wash	6.11	191	8855	45671	2725
P9	BC# 2 Plant 1	14.61	343	7893	861	2963
P10	BC# 2 Plant 1 Wash	3.24	297	2759	2522	1365
P11	BC# 2 Chara	42.04	429	2680	291	6178
P12	BC# 2 Chara Wash	13.29	260	1488	3586	368
P13	BC #2 Potpus	48.08	302	1427	861	1984
P14	BC #2 Potpus Wash	5.30	340	3492	1545	1336
P15	BC #2 nearshor crustose algae	9.56	228	2713	824	471
P16	BC #2 nearshor crustose algae Wash	4.59	208	1698	2825	326
P17	BC #2 potpus	31.06	273	1998	1023	1234
P18	BC #2 potpus Wash	10.37	230	1668	1156	665
P19	BC #2 dead cattail stock	4.07	221	2880	520	387
P20	BC #2 dead cattail stock Wash	BDL	182	2166	4612	250
P21	BC #2 dead cattail surface	BDL	153	892	614	256
P22	BC #2 dead cattail surface Wash	BDL	186	969	6064	242
P23	BC #1 cattail- Root	5.52	3920	1959	842	59794
P24	BC #1 cattail- Rhizo	5.43	1258	2044	728	24305
P25	BC #1 cattail- leaf	8.96	733	2375	418	36188
P26	BC #2 cattail- Root	11.98	1085	4490	1412	5993
P27	BC #2 cattail- Rhizo	4.79	450	3058	107	17369
P28	BC #2 cattail- leaf	7.24	48	3909	103	14156
P29	Dupe 1-BC #1	BDL	69	4318	11009	2380
P30	Dupe 2- BC# 2 Chara	28.93	316	1981	173	4817
P31	Dupe 3- BC #2 potpus	38.52	236	1084	489	1458
P32	Dupe 4- BC #2 cattail- leaf	9.06	55	4414	107	15336
P33	Dupe 5- BC #2 cattail- Root	15.51	1153	5294	3028	6332
P34	BC #3 Algae	6.28	395	1896	696	560
P35	BC #3 Algae Wash	BDL	666	9254	2853	1658
P36	BC #3 Cara Inflow	11.04	309	3615	3035	4874
P37	BC #3 Cara Inflow Wash	7.71	434	3755	3069	1828
P38	BC #3 Aquatic moss	95.49	298	2053	1544	2177
P39	BC #3 Aquatic moss Wash	7.26	350	3900	6793	540
P40	BC #3 Cara	14.16	184	3188	3388	2769
P41	BC #3 Cara Wash	16.29	211	3650	4363	2651
P42	BC #3 Stupee	189.30	881	3784	2593	13950
P43	BC #3 Stupee Wash	195.92	497	6137	5932	4545
P44	Cliff pond Potpus	42.33	1578	1900	286	10054
P45	Cliff pond Potpus Wash	BDL	180	522	281	383
P46	Cliff pond grassy plant	12.08	546	4679	1121	7802
P47	Cliff pond grassy plant Wash	BDL	313	5194	3561	2307
P48	Cliff pond weed #2	7.82	299	4815	954	3892
P49	Cliff pond weed #2 Wash	BDL	342	5309	2122	2823
P50	Cliff pond potnat	8.91	433	4937	854	4858
P51	Cliff pond potnat Wash	BDL	278	5264	1869	2294

		mg/kg dry mass				
	Sample	**B**	**Na**	**Mg**	**Al**	**K**
P52	Cliff pond algae bottom	7.52	177	5038	1735	652
P53	Cliff pond algae bottom Wash	BDL	152	4681	2801	1015
P54	Cliff pond floating algae	3.74	134	4191	5629	1471
P55	Cliff pond floating algae Wash	BDL	128	5503	22254	2538
P56	Cliff pond tire algae	34.86	190	3409	241	2808
P57	Cliff pond tire algae Wash	BDL	355	9582	7695	891
P58	Cliff pond- Root	BDL	2320	3715	395	27696
P59	Cliff pond- leaf	5.78	319	1395	17	14902
P60	Cliff pond plant - Root	8.91	6053	2927	854	15747
P61	Cliff pond plant - leaf	7.03	1370	6194	49	20846
P62	BC #1 Scripus- Root	5.73	1612	6526	1522	25587
P63	BC #1 Scripus- leaf	8.08	218	8469	430	23424
P64	Dupe 1-BC #3 Cara Inflow	9.57	265	3177	1718	4024
P65	Dupe 2- BC #3 Stupee	219.43	989	4231	1348	13976
P66	Dupe 3- Cliff pond potnat	8.82	505	5800	1040	5472
P67	Dupe 4- Cliff pond algae bottom	7.45	195	5858	2130	794
P68	Dupe 5- Cliff pond- leaf	4.19	393	1717	30	18702
P69	SRM 1 - TORT 2	3.89	12025	1081	9	8359
P70	SRM 2 -TORT 2	4.32	12541	1122	11	8480
P71	SRM 1 - TORT 2	11.56	13336	1196	15	8736
P72	SRM 2 -TORT 2	12.38	13901	1254	17	8943
	Average	**24.36**	**1259.47**	**3900.90**	**3697.15**	**7490.84**
	s.d	**45**	**2992**	**2236**	**6950**	**10153**
	Certified Value					
	MDL	2.75	2.48	4.11	3.55	5.10

Method- Microwave acid digestion ICP-MS

		mg/kg dry mass				
	Sample	**Ca**	**V**	**Cr**	**Mn**	**Fe**
P1	BC #1	1323	21	19	535	26133
P2	BC #1 Pond weed	6205	2	2	157	1564
P3	BC #1 Pond weed Wash	37143	19	16	569	17592
P4	BC #1 Before S. Curtain	1521	23	20	579	26535
P5	BC #1 Leaf Debris	11459	3	2	272	2244
P6	BC #1 Leaf Debris Wash	9121	17	13	499	13793
P7	BC #1 BSC Floating cattail Debris	33153	10	13	2111	6551
P8	BC #1 BSC Floating cattail Debris Wash	40545	18	13	1449	14826
P9	BC# 2 Plant 1	98468	1	1	1360	948
P10	BC# 2 Plant 1 Wash	130942	2	1	1625	1357
P11	BC# 2 Chara	138849	0	3	1189	541
P12	BC# 2 Chara Wash	141624	1	1	1846	1797
P13	BC #2 Potpus	161178	1	10	811	977
P14	BC #2 Potpus Wash	136934	2	1	1795	1469
P15	BC #2 nearshor crustose algae	163875	1	5	608	682
P16	BC #2 nearshor crustose algae Wash	159944	1	1	659	888
P17	BC #2 potpus	148153	1	4	1103	1162
P18	BC #2 potpus Wash	130814	1	1	1201	1414
P19	BC #2 dead cattail stock	139916	1	2	447	465
P20	BC #2 dead cattail stock Wash	153521	1	1	483	653
P21	BC #2 dead cattail surface	111234	1	2	436	1172
P22	BC #2 dead cattail surface Wash	139128	1	1	447	932
P23	BC #1 cattail- Root	2181	2	2	1288	4825
P24	BC #1 cattail- Rhizo	1577	1	2	175	3219

		mg/kg dry mass				
	Sample	**Ca**	**V**	**Cr**	**Mn**	**Fe**
P25	BC #1 cattail- leaf	2252	1	1	203	732
P26	BC #2 cattail- Root	33207	3	3	4979	34587
P27	BC #2 cattail- Rhizo	3199	0	0	262	2284
P28	BC #2 cattail- leaf	5073	0	1	810	925
P29	Dupe 1-BC #1	1384	17	15	472	22580
P30	Dupe 2- BC# 2 Chara	105416	0	2	903	421
P31	Dupe 3- BC #2 potpus	112384	1	6	605	703
P32	Dupe 4- BC #2 cattail- leaf	4898	0	1	894	959
P33	Dupe 5- BC #2 cattail- Root	39768	6	6	5929	39489
P34	BC #3 Algae	126197	1	1	27	433
P35	BC #3 Algae Wash	69892	2	2	108	1609
P36	BC #3 Cara Inflow	97667	4	6	2059	2469
P37	BC #3 Cara Inflow Wash	119403	4	3	1443	2286
P38	BC #3 Aquatic moss	110178	2	16	131	778
P39	BC #3 Aquatic moss Wash	127605	2	1	143	775
P40	BC #3 Cara	60798	4	7	2583	2313
P41	BC #3 Cara Wash	61351	6	4	1739	3857
P42	BC #3 Stupee	28048	4	5	1502	2262
P43	BC #3 Stupee Wash	44760	5	5	2542	6312
P44	Cliff pond Potpus	89418	0	3	72	198
P45	Cliff pond Potpus Wash	72382	0	0	43	172
P46	Cliff pond grassy plant	107259	2	6	254	855
P47	Cliff pond grassy plant Wash	139636	BDL	BDL	253	991
P48	Cliff pond weed #2	111190	1	3	249	779
P49	Cliff pond weed #2 Wash	132855	1	1	333	892
P50	Cliff pond potnat	88161	1	5	128	817
P51	Cliff pond potnat Wash	105301	2	1	217	1464
P52	Cliff pond algae bottom	95446	2	2	313	2222
P53	Cliff pond algae bottom Wash	84398	3	BDL	510	2637
P54	Cliff pond floating algae	54538	8	6	1441	7897
P55	Cliff pond floating algae Wash	38845	10	7	1306	11339
P56	Cliff pond tire algae	93659	0	1	85	287
P57	Cliff pond tire algae Wash	85061	3	3	262	756
P58	Cliff pond- Root	2681	2	2	136	2768
P59	Cliff pond- leaf	816	0	0	170	61
P60	Cliff pond plant - Root	5871	2	3	466	5762
P61	Cliff pond plant - leaf	4974	0	0	99	114
P62	BC #1 Scripus- Root	2030	3	6	1044	1917
P63	BC #1 Scripus- leaf	5155	1	6	637	711
P64	Dupe 1-BC #3 Cara Inflow	91040	2	4	1861	2092
P65	Dupe 2- BC #3 Stupee	34086	2	4	1706	2263
P66	Dupe 3- Cliff pond potnat	132319	1	6	148	959
P67	Dupe 4- Cliff pond algae bottom	130738	3	2	359	2399
P68	Dupe 5- Cliff pond- leaf	1221	BDL	0	204	73
P69	SRM 1 - TORT 2	2426	2	1	12	92
P70	SRM 2 -TORT 2	2488	2	1	13	95
P71	SRM 1 - TORT 2	2006	2	1	14	107
P72	SRM 2 -TORT 2	2100	2	1	15	112
	Average	**69033**	**4**	**4**	**852**	**4283**
	s.d	**55958**	**5**	**5**	**1031**	**7972**
	Certified Value		1.64	0.77	13.60	105.00
	MDL	40.64	0.14	0.18	0.40	1.86

Method- Microwave acid digestion ICP-MS

		mg/kg dry mass				
	Sample	Co	Ni	Cu	Zn	As
P1	BC #1	20.26	34.89	25.69	90.93	5.26
P2	BC #1 Pond weed	6.38	19.47	26.24	101.28	BDL
P3	BC #1 Pond weed Wash	18.29	39.64	46.94	184.16	BDL
P4	BC #1 Before S. Curtain	21.49	35.76	26.09	94.83	5.63
P5	BC #1 Leaf Debris	7.50	20.74	11.39	55.60	BDL
P6	BC #1 Leaf Debris Wash	15.09	48.88	49.05	132.84	BDL
P7	BC #1 BSC Floating cattail Debris	32.33	68.39	33.02	110.89	2.22
P8	BC #1 BSC Floating cattail Debris Wash	27.77	41.22	17.75	133.65	3.54
P9	BC# 2 Plant 1	1.73	6.98	3.41	16.06	BDL
P10	BC# 2 Plant 1 Wash	1.66	7.22	3.14	21.69	BDL
P11	BC# 2 Chara	1.83	13.03	3.24	17.61	BDL
P12	BC# 2 Chara Wash	2.32	10.17	3.87	20.36	BDL
P13	BC #2 Potpus	1.73	14.09	3.95	12.50	BDL
P14	BC #2 Potpus Wash	1.87	9.59	2.37	13.31	BDL
P15	BC #2 nearshor crustose algae	1.47	12.95	13.99	11.92	BDL
P16	BC #2 nearshor crustose algae Wash	1.55	9.76	2.62	11.11	BDL
P17	BC #2 potpus	1.89	12.69	1.83	12.78	BDL
P18	BC #2 potpus Wash	1.59	8.83	3.46	11.69	BDL
P19	BC #2 dead cattail stock	1.22	10.32	7.18	11.95	BDL
P20	BC #2 dead cattail stock Wash	1.34	9.28	1.47	16.50	BDL
P21	BC #2 dead cattail surface	1.27	7.81	2.59	7.15	BDL
P22	BC #2 dead cattail surface Wash	1.43	8.27	1.71	8.72	BDL
P23	BC #1 cattail- Root	50.51	11.86	27.66	65.77	3.38
P24	BC #1 cattail- Rhizo	6.88	3.51	10.77	33.04	BDL
P25	BC #1 cattail- leaf	1.19	2.35	8.91	22.85	BDL
P26	BC #2 cattail- Root	34.89	64.23	10.51	68.48	3.07
P27	BC #2 cattail- Rhizo	1.89	4.10	2.69	23.05	BDL
P28	BC #2 cattail- leaf	0.70	2.26	2.57	10.80	BDL
P29	Dupe 1-BC #1	17.84	30.43	22.17	79.03	4.72
P30	Dupe 2- BC# 2 Chara	1.45	10.51	2.38	12.54	BDL
P31	Dupe 3- BC #2 potpus	1.35	10.79	2.86	9.87	BDL
P32	Dupe 4- BC #2 cattail- leaf	0.72	2.41	2.67	12.36	BDL
P33	Dupe 5- BC #2 cattail- Root	38.95	71.55	11.95	79.66	3.47
P34	BC #3 Algae	0.83	7.86	2.47	13.85	BDL
P35	BC #3 Algae Wash	1.27	6.26	5.19	20.53	BDL
P36	BC #3 Cara Inflow	3.94	18.44	3.31	33.76	BDL
P37	BC #3 Cara Inflow Wash	3.21	14.77	5.37	25.22	BDL
P38	BC #3 Aquatic moss	1.68	20.75	6.86	13.58	BDL
P39	BC #3 Aquatic moss Wash	1.67	12.28	2.42	15.20	BDL
P40	BC #3 Cara	2.66	19.88	4.78	40.75	BDL
P41	BC #3 Cara Wash	3.42	18.78	6.74	34.38	BDL
P42	BC #3 Stupee	2.89	14.76	9.14	51.73	BDL
P43	BC #3 Stupee Wash	6.10	15.72	39.52	70.82	BDL
P44	Cliff pond Potpus	0.77	8.10	2.12	15.00	BDL
P45	Cliff pond Potpus Wash	0.48	3.94	1.38	4.70	BDL
P46	Cliff pond grassy plant	1.60	13.44	26.51	44.83	BDL
P47	Cliff pond grassy plant Wash	BDL	BDL	BDL	79.52	BDL
P48	Cliff pond weed #2	1.48	12.88	2.91	38.58	BDL
P49	Cliff pond weed #2 Wash	1.67	10.73	3.33	34.29	BDL
P50	Cliff pond potnat	1.39	10.06	9.06	25.27	BDL
P51	Cliff pond potnat Wash	1.83	9.65	5.24	32.17	BDL
P52	Cliff pond algae bottom	2.47	10.97	2.56	23.95	BDL
P53	Cliff pond algae bottom Wash	3.66	14.68	5.05	43.80	BDL
P54	Cliff pond floating algae	7.98	20.03	9.28	54.98	2.36
P55	Cliff pond floating algae Wash	10.72	26.61	12.76	84.46	BDL
P56	Cliff pond tire algae	0.89	7.46	2.11	18.45	1.73

		mg/kg dry mass				
	Sample	Co	Ni	Cu	Zn	As
P57	Cliff pond tire algae Wash	3.39	18.08	8.06	63.98	21.43
P58	Cliff pond- Root	5.75	7.26	6.07	45.85	7.59
P59	Cliff pond- leaf	0.22	1.31	4.28	13.77	1.61
P60	Cliff pond plant - Root	10.07	34.50	23.06	282.09	12.13
P61	Cliff pond plant - leaf	0.26	1.64	3.89	23.92	BDL
P62	BC #1 Scripus- Root	12.61	36.17	45.12	88.67	2.56
P63	BC #1 Scripus- leaf	2.33	7.99	46.09	82.57	2.30
P64	Dupe 1-BC #3 Cara Inflow	3.73	17.26	3.32	31.70	1.96
P65	Dupe 2- BC #3 Stupee	3.43	17.35	11.51	61.46	2.04
P66	Dupe 3- Cliff pond potnat	1.58	11.47	12.14	29.10	BDL
P67	Dupe 4- Cliff pond algae bottom	2.65	11.71	2.53	26.04	BDL
P68	Dupe 5- Cliff pond- leaf	BDL	BDL	4.65	14.07	BDL
P69	SRM 1 - TORT 2	0.46	2.07	96.10	181.32	21.54
P70	SRM 2 -TORT 2	0.50	2.18	99.57	188.69	22.36
P71	SRM 1 - TORT 2	0.56	2.45	109.35	200.51	23.84
P72	SRM 2 -TORT 2	0.57	2.54	113.71	208.34	24.78
	Average	**6.27**	**16.23**	**16.25**	**53.85**	**8.16**
	s.d	**10**	**15**	**25**	**57**	**8**
	Certified Value	**0.51**	**2.50**	**106.00**	**180.00**	**21.60**
	MDL	**0.15**	**0.71**	**0.30**	**1.27**	**1.40**

Method- Microwave acid digestion ICP-MS

		mg/kg dry mass				
	Sample	Sr	Mo	Cd	Ba	Pb
P1	BC #1	38	BDL	BDL	128.09	19.94
P2	BC #1 Pond weed	175	BDL	BDL	19.84	1.67
P3	BC #1 Pond weed Wash	1014	BDL	BDL	123.08	22.11
P4	BC #1 Before S. Curtain	37	BDL	BDL	130.47	20.04
P5	BC #1 Leaf Debris	286	BDL	BDL	72.99	4.31
P6	BC #1 Leaf Debris Wash	183	BDL	BDL	98.71	24.83
P7	BC #1 BSC Floating cattail Debris	509	BDL	0.61	65.87	7.43
P8	BC #1 BSC Floating cattail Debris Wash	567	BDL	BDL	103.48	16.60
P9	BC# 2 Plant 1	3161	BDL	BDL	36.56	0.57
P10	BC# 2 Plant 1 Wash	4209	BDL	BDL	48.60	1.99
P11	BC# 2 Chara	3848	BDL	BDL	46.61	0.19
P12	BC# 2 Chara Wash	4107	BDL	BDL	49.82	2.81
P13	BC #2 Potpus	4443	BDL	BDL	45.59	0.48
P14	BC #2 Potpus Wash	3874	BDL	BDL	47.44	1.06
P15	BC #2 nearshor crustose algae	3990	BDL	BDL	40.34	0.50
P16	BC #2 nearshor crustose algae Wash	4093	BDL	BDL	41.85	1.44
P17	BC #2 potpus	4148	BDL	BDL	45.69	0.66
P18	BC #2 potpus Wash	3652	BDL	BDL	41.59	1.19
P19	BC #2 dead cattail stock	3441	BDL	BDL	32.90	1.05
P20	BC #2 dead cattail stock Wash	3903	BDL	BDL	37.75	1.96
P21	BC #2 dead cattail surface	3174	BDL	BDL	31.72	0.49
P22	BC #2 dead cattail surface Wash	4050	BDL	BDL	39.99	1.46
P23	BC #1 cattail- Root	60	BDL	BDL	18.74	13.79
P24	BC #1 cattail- Rhizo	40	0.66	BDL	12.89	3.32
P25	BC #1 cattail- leaf	47	BDL	BDL	8.94	0.76
P26	BC #2 cattail- Root	625	BDL	BDL	76.20	2.55
P27	BC #2 cattail- Rhizo	66	BDL	BDL	2.81	0.26
P28	BC #2 cattail- leaf	102	BDL	BDL	3.84	BDL
P29	Dupe 1-BC #1	34	BDL	BDL	116.35	16.85
P30	Dupe 2- BC# 2 Chara	2990	BDL	BDL	36.97	BDL

		mg/kg dry mass				
	Sample	Sr	Mo	Cd	Ba	Pb
P31	Dupe 3- BC #2 potpus	3448	BDL	BDL	35.11	0.35
P32	Dupe 4- BC #2 cattail- leaf	109	BDL	BDL	3.89	BDL
P33	Dupe 5- BC #2 cattail- Root	831	0.57	BDL	88.13	3.04
P34	BC #3 Algae	5291	BDL	BDL	49.33	0.47
P35	BC #3 Algae Wash	2679	BDL	BDL	34.34	1.82
P36	BC #3 Cara Inflow	3330	BDL	BDL	48.74	1.95
P37	BC #3 Cara Inflow Wash	4839	BDL	BDL	52.55	2.12
P38	BC #3 Aquatic moss	4497	BDL	BDL	41.84	0.73
P39	BC #3 Aquatic moss Wash	5333	BDL	BDL	51.85	1.94
P40	BC #3 Cara	1783	BDL	BDL	43.23	2.06
P41	BC #3 Cara Wash	1823	BDL	BDL	42.61	3.03
P42	BC #3 Stupee	858	BDL	BDL	26.09	1.75
P43	BC #3 Stupee Wash	1457	BDL	BDL	55.17	6.61
P44	Cliff pond Potpus	650	BDL	BDL	69.72	0.18
P45	Cliff pond Potpus Wash	534	BDL	BDL	57.67	0.25
P46	Cliff pond grassy plant	549	BDL	BDL	76.42	0.92
P47	Cliff pond grassy plant Wash	683	BDL	BDL	96.12	BDL
P48	Cliff pond weed #2	509	BDL	BDL	73.92	0.74
P49	Cliff pond weed #2 Wash	651	BDL	BDL	93.23	1.83
P50	Cliff pond potnat	495	BDL	BDL	76.99	0.81
P51	Cliff pond potnat Wash	508	BDL	BDL	93.85	2.49
P52	Cliff pond algae bottom	434	BDL	BDL	67.10	2.06
P53	Cliff pond algae bottom Wash	377	BDL	BDL	69.12	3.61
P54	Cliff pond floating algae	242	0.88	BDL	74.08	6.54
P55	Cliff pond floating algae Wash	155	BDL	BDL	84.97	12.72
P56	Cliff pond tire algae	518	0.57	0.69	63.45	0.59
P57	Cliff pond tire algae Wash	358	6.88	9.29	60.64	6.44
P58	Cliff pond- Root	26	2.33	2.78	10.30	5.00
P59	Cliff pond- leaf	4	0.90	0.69	1.29	0.23
P60	Cliff pond plant - Root	40	1.45	3.15	43.97	3.02
P61	Cliff pond plant - leaf	27	0.54	BDL	4.61	0.22
P62	BC #1 Scripus- Root	51	1.04	1.47	16.71	2.79
P63	BC #1 Scripus- leaf	129	1.38	1.22	12.49	0.93
P64	Dupe 1-BC #3 Cara Inflow	2999	0.54	0.67	41.84	1.87
P65	Dupe 2- BC #3 Stupee	984	0.62	0.84	24.80	1.95
P66	Dupe 3- Cliff pond potnat	572	BDL	BDL	86.72	0.89
P67	Dupe 4- Cliff pond algae bottom	464	BDL	BDL	69.93	2.05
P68	Dupe 5- Cliff pond- leaf	3	BDL	BDL	BDL	BDL
P69	SRM 1 - TORT 2	43	0.93	26.12	1.53	0.38
P70	SRM 2 -TORT 2	44	0.99	27.04	1.65	0.38
P71	SRM 1 - TORT 2	47	1.14	29.06	1.93	0.44
P72	SRM 2 -TORT 2	49	1.18	29.66	1.94	0.41
	Average	**1518**	**1.33**	**9.52**	**50.08**	**3.82**
	s.d	**1722**	**1**	**12**	**33**	**6**
	Certified Value	**45.20**	**0.95**	**26.70**		**0.35**
	g	**0.89**	**0.49**	**0.61**	**0.70**	**0.17**

Method- Microwave acid digestion ICP-MS   BDL: Below Detection Limit

7. Sulphur concentrations in water and biomass

Water samples

Total Sulfur	mg/L
BC1 outflow	3250
BC2	3510
BC2 inflow	6990
BC2 outflow	5940
BC3	6060
BC3 inflow	7150
cliff pond 28	3430
cliff pond outflow	6020
H2O BC1 ASC	2690
H2O BC1 BSC	3110
inflow cliffs	4090

Method- ICP-AES

Chara samples

	% Sulfur
1-BC#2 Chara	0.37
2-BC#2 Chara wash	0.28
3-BC#3 Cara inflow	0.53
4-BC#3 Cara inflow wash	0.82
5-BC#3 Cara	0.59
6-BC#3 Cara wash	0.68

Method- Leco combustion elemental analyzer

Do mining effluent ponds with charophytes share distinctive physical, chemical and biotic characteristics?

Margarete Kalin[1], Robin Scribailo[2], William N. Wheeler[1] and G. Meinrath[3]

[1]Boojum Research Ltd., Toronto, Ontario Canada

[2]Biological Sciences, Purdue North Central University

[3]RER Consultants Fuchsbauerweg 50 94036 Passau Germany

Abstract:

Charophytes are early colonizers of lakes and ponds affecting water clarity and the chemical composition of both water and sediments. We investigated underwater meadows of *Chara vulgaris* and *Nitella flexilis* in alkaline mine waste waters. The habitats covered pit lakes and shallow ditches and ponds, constructed of tailings (ground, fine mine wastes) in which mine waste seepage collects. The gold, zinc, nickel, uranium and coal mines are located from the boreal shield of Canada to the mid-latitude deciduous forests in the U.S.A. We also sampled uncontaminated lakes in Ontario, Canada.

We characterized the physical dimensions of the water bodies along with measurements of pH, electrical conductivity and E_h. Biomass was characterized by the height of plants, % cover estimates and standing crops. Sediments were described using pH, electrical conductivity, E_h, oospore density, and 'loss on ignition.' Water, sediment and biomass samples were also analyzed by ICP for a range of elements. All of these parameters went into a blind statistical data set and analyzed using correlations, PCA and factor analysis.

None of the measured parameters could be related to the standing biomass. The elemental concentrations in water and sediment showed large variations with no significant or valid correlations in these data sets. However, for both *C. vulgaris* and *N. flexilis* biomass, the elemental concentrations displayed statistically significant correlations among certain groups of elements, regardless of the elemental concentrations in the water. Four groups of biomass / water concentrations were compared (uncontaminated, gold, uranium and 'other mines' mine effluents. Although the biomass data set is an empirically-random set of samples, a remarkable pattern emerged when the elemental enrichment in the biomass was evaluated for different, chemically-related elements. The alkali metals, K and Na, were consistently accumulated. Ba, Ca, Mg and Sr (alkali earth metals) along with the transition metals (Cu, Fe, Mn, Ni and Zn) were also accumulated, probably by co-precipitation, given the prevailing carbonate geochemistry of the water and the reactivity of the characean cell wall. The post transition metals Al, As, B and the non-metals and actinoids, Se, P, S and U did not follow a regular pattern. However, while the concentration of sulphur ranged between 10 to 1000 mg L^{-1}, its concentration in the biomass of all groups was around 10,000 mg kg^{-1}. We conclude that its role as a growth-controlling factor should be addressed.

The elemental concentrations in this data set of *Chara vulgaris* and *Nitella flexilis* biomass and water revealed a strong biogeochemical component reflecting the elemental composition of the characean cell wall. The unique cell wall characteristics of charophytes probably mask many of the presently considered parameters necessary for growth. A systematic experimental approach is needed to clarify the role of sulphur and the carbonate biogeochemistry on the cell wall. A re-assessment of bio-and hyper-accumulation terminology may be needed since differences may be due to the uniqueness of the characean cell wall and its ability to influence biogeochemical reactions.

1.0 Introduction

Charophytes occupy a wide range of aquatic environments where light penetration, water depth, hydrological regimes and the nutrient status of the water are considered relevant growth-controlling factors (van der Berg *et al*. 1999; Coops, 2002). Charophytes are important components of lake ecosystems that are well known to contribute to water quality primarily because of their ability to enhance water clarity (Scheffer, 1992) and remove a variety of elements from the water by exchange processes (e.g. McConnaughey, 1998; McConnaughey and Falk 1991). In the latter case, characean populations modulate the pH of the water around the cell walls, and during intensive growth these plants can alter the chemistry of the water (Ray *et al*. 2003). The ability of charophytes to utilize carbonic anhydrase in charosomes enables these algae to photosynthesize in high pH waters, while accumulating inorganic carbon encrustations on the outer cell membrane (Arancibia-Avila *et al*. 2001). By changing the pH of the surrounding water and by precipitating calcium carbonate, these algae affect processes such as adsorption, absorption, chelation, precipitation and the co-precipitation of a variety of elements (Pelechaty *et al*., 2013). Characean biomass can serve as nutrients sinks (Kufel and Kufel, 2002; Rodrigo *et al*., 2007), and through the process of apical growth and basal decay form a rich organic sediment. Such sediments support bio-mineralization in a reducing, microbially-active sediment, stabilizing inorganic elements, a process which can lead to the formation of biogenic ore bodies. This process is well-documented for uranium and zinc ore bodies (Culbert, 1984, McConnaughey, *1999;* Warren *et al.,* 2001; Kalin *et al*. 2005, Freytet and Verrecchia 1998). The formation of carbonate deposits in pre-Quaternary environments documents all stages of the fossilization process highlighting the formation of oolithic carbonate rocks (Khanaqa *et al.,* 2012).

Biogeochemical prospecting for metals and phytoremediation of soils and waste water both utilize the capacity of plants to accumulate metals. Hyper-accumulators are defined as plants minimally containing between 0.01% and 1% dry weight of an element (Prasad and Freitas, 2003; Clabeaux *et al*., 2011). Utilizing these concentration differences between water and biomass the charophytes are hyper-accumulating algae for many elements (Gomes and Asaeda 2010). Various authors have suggested the potential of charophytes for use in bioremediation particularly where efforts are being made to reduce levels of toxic elements in waste streams (Marquardt and Schubert, 2009; Gomes and Asaeda 2010). On a world-wide basis the magnitude of the problem caused by these waste streams cannot be understated in terms of the extent of contamination of habitat and risk to organismal health at every trophic level. Added difficulties are engendered by the scarcity of adequate cost-effective treatment systems, which can deal with the perpetuity of contaminant generation through weathering processes, particularly prevalent with mining wastes. Bioremediation utilizes biological processes such as growth, adsorption, absorption and uptake in rooted plants to remove contaminants from the soil. The plants are harvested and need to be processed if they have accumulated metals. In the water bioremediation processes are utilized to remove the contaminant from the water and relegated the inorganic metals to the sediments where they are stabilized there through microbially-driven bio-mineralization processes. The annual re-growth of biomass generates new reactive surfaces for contaminant removal, and thus offers a cost-effective and environmentally-sustainable treatment option for waste water or as an addition or alternative to expensive, physical, chemical treatment methods (Kalin *et al.,* 2007).

Several ecological processes have been developed for the treatment of acidic and alkaline mine waste water during our work on mine waste management areas in Canada, the United States, and Germany. We found that *Chara vulgaris* and *Nitella flexilis* populations thrive in many heavy metal and radionuclide-laden circum-neutral mine waste waters. This paper summarizes some of our work on charophytes, along with the physical and chemical parameters of the mine waste water ponds, pit-lakes and ditches where we observed the populations of both species intermittently. Specific questions addressed in this paper include: 1) Does an analysis of the physical and chemical characteristics of mine waste water habitats, spread over a wide geographical area, reveal any ecologically-relevant similarities? 2) Is the elemental profile of the water and the sediment indicative of concentrations found in biomass? 3) Are there significant correlations among the elements in the biomass which might suggest that they are of relevance to growth. The answers to these questions should provide valuable insights that will facilitate the development of a more extensive role for charophytes in bioremediation systems.

2 **Methods and Materials**

2.1 **Site descriptions**

The data sets include records from gold, uranium, nickel and coal mines, as well as natural, clean lakes and ponds. Most of the mine sites and uncontaminated water bodies studied are located on the Boreal Shield which stretches from Northern Saskatchewan to northern Ontario. One control site and one nickel mine lie in the Hudson Plains which reach into Central Ontario (South Bay and Falconbridge). Lake St. Clair is located in the mixed wood plains in Southwestern Ontario. The coal mines in W. Virginia are located in the mid-latitude deciduous forest. Observations were carried out intermittently during the summer when projects were running in the vicinity and at least once during the winter by drilling through the ice to ascertain presence or absence of the populations in Northern Ontario and Saskatchewan. *C. vulgaris* and *N. flexilis* biomass proliferated throughout summer and winter months at all gold mines and Lower Link Lake in Northern Saskatchewan. For the other mine sites, where *C. vulgaris* occurred, winter observations were not made. Populations of *C. vulgaris* in the valley seep ponds were populated with other submerged and emergent vegetation. *C. vulgaris* biomass was only sampled twice, once in the fall and spring.

Nitella flexilis was studied extensively over a 12 year period from 1988 through 2000. During the 12 year study period, the population in Lower Link Lake collapsed once during the winter, due to a break in the beaver dam at the outflow of Lower Link Lake controlling its water level. The ice cover crushed and scoured the *N. flexilis* biomass. The population completely recovered by the second growing season.

2.2 **Sample collection and preparation**

Biomass was collected between 1980 and 1999 in the gold mining district Timmins, Northern Ontario and again in 2001 – 2002. At this time collections from nearby uncontaminated lakes were also made. *C. vulgaris* populations in the W. Virginia valley fills were only sampled twice, once in October of 2012 and once in June of 2013. The standing biomass was collected using a large rake which removed 0.5 m^2 biomass in at least 3 locations at each site. At randomly-selected sites in lakes, ponds or ditches, 3 to 5 Eckman grab samples of sediments were collected.

The collected biomass samples were rinsed thoroughly *in situ* to wash off any sediment particles and all epiphytic growth visible by the naked eye. Large pieces of debris were removed by hand. The samples were then transported in plastic bags in coolers. In the laboratory, the plants and the sediments were dried in an oven at 60^o C until a steady weight was obtained. The dried material was subsampled with one quarter either ground in a Wiley vegetation mill or in a hand mortar. Sediment was all ground in a hand mortar. The powder was again dried in weighing boats for 24 h to constant weight in the drying oven at the same temperature. One gram of well-mixed powder from the ground samples was subsampled and submitted for wet digestion to a certified laboratory (Saskatchewan Research Council, Inorganic Analytical Laboratory, Saskatoon, Canada). A second quarter was also oven-dried in the same manner, but then ‘burned’ at 450^{o} C to determine the ash weight or ‘Loss on Ignition’ (LOI). This value is a crude estimate of organic matter (Allen, 1974).

2.3 Physical and chemical parameters of the water

The redox potential was measured using a Corning 102 pH meter with a Fisher calomel electrode and an Orion platinum electrode. E_m (measured redox potentials) readings were converted to E_h after calibration with standard redox buffer. The pH was measured using either a Corning 102 pH meter with a Canlab combination pH electrode or a WTW 196 meter. Both meters were used in combination with either single or double point calibration. Conductivity was measured with an Orion (WTW) Model 140 meter. Measurements of pH, E_h, and electrical conductivity were carried out in the field and/or in the laboratory following Rand *et al.* (1976). The nutrient status of the water was determined for the water of the uncontaminated lakes and the gold and nickel sites. Phosphate (PO_4) was below the detection limit < 0.1 mg L^{-1} in all waters with the exception of Langmuir (0.2 mg L^{-1}). NO_3 was below the detection of 1 mg L^{-1} as determined with color spectroscopy by HACH™.

Water samples were collected in 500 mL plastic bottles that were placed immediately in coolers and shipped within 36 hours to the laboratory. Samples for metal analysis were filtered through 0.45 µm membrane filters, acidified with nitric acid to < pH 1, sent to a certified laboratory and analyzed by Inductively Coupled Plasma Spectroscopy (ICP; US EPA method #200.7). One gram of the biomass and sediment subsamples were digested in concentrated nitric or perchloric acid to determine elemental concentrations, also with ICP.

2.4 Data selection and statistical treatment

The data set consisted of 24 water samples and 24 biomass samples. At each *Chara vulgaris* sampling event and location, at least one biomass and water from the same date (or within 2 days) were collected. The *Nitella flexilis* biomass and water samples consisted of 8 water and 10 biomass samples all from the same lake, but sampled over several years.

The selection of elements for presentation was based on their analytical detection limit, i.e. an elemental concentration in the biomass had to be at least 2 x the reported detection limit. For example, Cr had detection limits in the biomass which ranged from 2 to 212 mg kg^{-1}. Chromium concentrations never rose above 4 or 424 mg kg^{-1}, so it was not considered. This criterion was needed, as the analytical methods, and with this, the analytical detection limits in complex elemental matrixes, decreased over the last 2 to 3 decades in which the data were obtained. The elements Ag, Be, Co, Cr, Pb, Sn, Ti, V and Zr were at or below the detection limits given in Supplemental Table S1.

The elemental concentrations of water, sediment and biomass, respectively, were collated into data matrices. Missing elemental concentrations were replaced by either mean values (if less than five data points were available in the other rows of the data matrix) or random samples from the empirical probability distributions (if more than five data points were available in the row). Data analysis proceeded by the following successive steps: a) generation of correlation matrix; b) assuring statistical significance of the variations in the correlation matrices; c) principal component analysis (PCA); d) estimation of the number significant Eigen vectors; and e) determination of the factor structure to identify the factors giving rise to the observed correlation matrix. This sequence is a standard procedure in multivariate data analysis. Further details are given in (Catell, 1966); Golub and Reinsch, 1970; Cooley and Lohnes, 1971; Morrison, 1976). Statistical significance was tested by Kolmogoroff-Smirnov- and Bartlett's sphericity tests (Chakravarti *et al*., 1967; Snedecor and Cochran, 1989).

3. Results

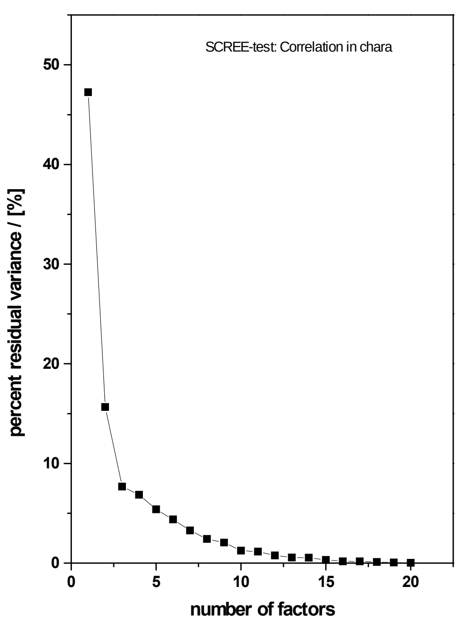
3.1 **Statistical considerations and data validation**

A multi-variable correlation of all water in which *Chara vulgaris* biomass was sampled revealed, among the relatively small number of samples, a large variation in concentrations. No significant trends could be detected (concentration ranges presented in supplementary data section (Supplemental Table S2). This is not surprising as the water originated from different mining wastes and uncontaminated environments, in varying climatic geological regions. Consequently, Bartlett's sphericity test indicated that variations in the correlation matrix are insignificant. Further analysis of the water sample matrix therefore ceased. The same result was obtained for the data matrix holding element concentrations in the sediments for those sites where sediments are available. (Supplemental Table S3). However, for the matrix of the elemental concentrations of the biomass a significant correlation was found over all sites. This was not expected as the variation in the water concentration represented a random association of elements above the detection limit. For the *Nitella flexilis* data set no statistical tests were performed as these samples were from one location sampled over several years. Because conditions had not changed, no differences among the data sets were expected.

The biomass matrix was manipulated as a random assembly of *Chara vulgaris* biomasses, where the plants grew in uncontaminated and mining waste waters. The question we posed was, do the elemental compositions of these plants reflect the different elemental compositions of the waters? Are there any other elements (besides carbon, nitrogen and phosphate compounds (see Box, 1987, 1988; Rodrigo *et al*., 2007) which might be needed to sustain growth? Multivariate correlations provide some interesting observations. Small negative correlation coefficients (-0.6) are present indicated by red fields (Supplemental Table S4) in the upper and lower triangular matrices mainly for Ni, Sr, Zn and Cu (all toxic elements at elevated concentrations). High correlation coefficients (> 0.75; indicated in yellow in Supplemental Table S4) were scarce but occurred for K, Na, Bi, B, Al, S, Si and U. For these elements, however, the coefficients may be artificial as those elements are not always present in the matrix, as they were not included in the earlier ICP analyses. On the other hand, with the exception of Al, Bi and U, all these elements are known algal micronutrients.

Factor analysis was then performed. The square correlation matrix was broken down into orthogonal or independent vectors, eigenvectors and eigenvalues. Since the eigenvalue represents the total variance explained by each factor, the homogeneity of variances between the elements in the biomass was analyzed by Bartlett's Sphericity test. To interpret the importance of each factor, a graphical presentation in the form of the SCREE test was generated, which essentially ranks the factors according their contribution to the variance (Figure 1). The shape of the curve is nearly classical in that the first factors dominate the variance and the remaining factors mainly represent random noise in the data. The SCREE, a standard way to represent the information of each eigenvector, indicated three significant eigenvectors, thereby suggesting the remaining eigenvectors represent mainly statistical noise. This noise can be safely neglected during the subsequent data analysis.

Figure 1: SCREE test results for factor analysis of *Chara vulgaris* biomass and water



To identify the elements comprising each of the three factors, the factor structure was determined. The first, and main, elemental factors in the *C. vulgaris* biomass were Ca, Mg, and P followed by the second factor with Ba and Sr and notably, the transition elements Ni and Cu. If the Ca content in biomass is low then Ni is negatively correlated with the majority of the other 18 elements in the biomass matrix. These relationships are considered statistically sound, and suggest that the uptake of Ni by the biomass may depend on the availability of calcium. For the third factor, no clear information concerning the elements could be obtained.

3.2 **Physical characteristics of the habitats**

The habitats in which *Chara vulgaris* and *Nitella flexilis* represented the dominant biota varied widely. In the case of mine waters, *C. vulgaris* was dominant in shallow ditches at the foot of gold tailings dams or in shallow pools on the top of tailings dams (Hollinger, Kidd Creek, Schumacher 1 and 2, Falconbridge and South Bay; Table 1). These systems experience large temporal fluctuations in water level which intermittently expose the upper thalli of the biomass. *Chara vulgaris* was also found in lakes formed in former open pits, which were filled with ground water from underground mining tunnels and atmospheric precipitation when mining ceased (Langmuir nickel mine and Little Pearl Lake gold mine). Little Pearl Lake has no littoral zone as it is a steep-walled open pit, whereas Langmuir served as a tailings depository and the finely-ground rock (tailings) created a sandy, shallow bench. *Chara vulgaris* was also found in small shallow ponds at the foot of steep rock valley fills, created by mountain-top coal mining. These valley fills were created when coal seams were removed from mountain tops and the overburden was used to fill in existing valleys.

Of course, *Chara vulgaris* was also found in uncontaminated natural kettle lakes, such as Middle Triple and Irrigation Lake (Northern Ontario, Canada). The shores of the large, natural Lake St. Clair in Southern Ontario (Canada) also supported a dense, littoral population of *C. vulgaris*. *Nitella flexilis* populated the Link Lake system (Northern Saskatchewan, Canada), where it formed a monoculture in Lower Link Lake. A sedimentation dam separates Upper from Lower

Link Lake. The upper lake was used to retain a one-time release of sediments from the Rabbit Lake open pit. This sudden release of the sediment destroyed all macrophytes in Upper Link Lake, increasing the concentration of Ra226 in the lake effluent. The mining company engaged us to provide a biological solution to the elevated radionuclide problem. We transplanted *N. flexilis* to the Upper Link Lake in 1989. With the expansion of the *N. flexilis* population in the Upper Link Lake, the concentration of Ra226 in the Upper Link Lake again dropped. The decrease in Ra226 concentration was attributed to the dense monoculture of *N. flexilis.*

In Table 1, the physical dimensions, chemical and physical characteristics of the water bodies and the observed characean cover are presented. The lake or water body sizes ranged from 39 to 0.5 ha, with algal covers of 100% to very sparse (below 1%) in two control lakes, Middle Triple lake and Irrigation Lake. The water depth ranged from 0.5 to 1 m in the ditches and 4.5 to 7.4 m in the lakes. The height of the plants ranged from about 0.1 to 1 m. The 100% cover values for the shallow lakes and ditches in the Timmins area and the Link lakes are very reliable estimates as they were provided by aerial surveys of the lakes in 1985. Visual determinations of cover in the ditches over several years of observations are also reliable.

Table 1: *Chara* and *Nitella* study locations: water pH, E_h, Electrical conductivity 2001-02 and 2012-13

Location	Area	Depth	Cover	Height	pH	Eh	Cond.
	ha	m	%	m		mV	µS cm^{-1}
Northern Saskatchewan (58° 11’ N, 103° 41’ W)							
Upper Link lake	34	0.9-1.7	80	0.4	7.5	452	150

Lower Link lake	39	0.6-1.9	100	0.8	7.0	437	95
Timmins, Northern Ontario (48° 35′ N, 81 ° 52 ′ W)							
Hollinger	24	0.6	100	0.3	8.0	n.a.	190
Irrigation Lake	7	8-10	1	0.2	8.0	556	119
Kidd Creek	n.d.	0.5	100	0.5	9.0	n.d.	2100
Langmuir	7	0.8	50	0.2	7.7	551	544
Little Pearl Lake	0.5	7.4	50	0.4	7.4	543	2850
Middle Triple Lake	3	1	1	0.3	7.4	394	121
Pamour	3.3	0.5	100	0.1	8.2	n.d.	230
Schumacher 1	1	6.8	100	0.4	6.8	458	1330
Schumacher 2	0.5	0.5	100	0.2	7.2	502	2480
Sudbury, Northern Ontario (46° 27' N, 81o 7' W)							
Falconbridge	n.d.	1-4.5	80	0.4	9.0	n.d.	2100
Southwestern Ontario (42° 22′ N, 82° 22′ W)							
St. Clair	4	0.5-2	10	0.2	7.5	394	232
Northwestern Ontario (51° 6' N, 92o 40' W)							
South Bay	n.d.	0.5-1	100	0.3	6.9	400	750
West Virginia, USA (37° 47' N, 81o 50' W)							
Valley Fill 1a CM1	n.d.	n.d.	n.d.	n.d.	7.0	121	1342
Valley Fill 1b CM2	n.d.	n.d.	n.d.	n.d.	7.3	58.4	1316
Valley Fill 2a HB1	n.d.	n.d.	n.d.	n.d.	7.2	76.9	194
Valley Fill 2b HB2	n.d.	n.d.	n.d.	n.d.	7.0	145	468
West Virginia, USA (38° 2' N, 81o 44' W)							
Valley Fill 3 BC2	0.17	2.5	2	n.d.	7.3	n.d.	3310
Valley Fill 4a BC3	0.64	1-7	50	n.d.	7.7	n.d.	3324
Valley Fill 4b BC3	0.64	1-7	50	n.d.	7.7	n.d.	3324

The pH ranged from 6.9 to 9.0. All water displayed a positive redox value ranging between 394 to 556 mV, well within the normal range of surface waters. However, the charophytes studied grew in waters with a wide range of electrical conductivities. Dense populations of charophytes grew in nearly single-distilled water with a conductivity as low as 95 $\mu S\ cm^{-1}$ (Link lakes) to waters with conductivities that exceeded 2500 $\mu S\ cm^{-1}$ (gold, zinc and coal mine seepages).

3.3 **Physical and biological characteristics of sediments of both genera**

In Table 2, the sediment characteristics are presented for those sites where previously oospores were collected (Kalin and Smith 2007), together with two indicators of plant growth, oospore density and standing biomass. The organic content of the sediments (LOI) varied greatly from 60% organic (Link lakes) to 1% (Middle Triple Lake). The sediment pH values, as expected, were neutral, similar to the water above. Even though there were large differences in organic matter content, the redox values (E_h) were all negative, suggesting the sediments were 'reducing' with low oxygen concentrations. The electrical conductivities of the sediments did not reflect the same wide range as the water. Either the conductivities were similar or lower than the water. For example, Schumacher 2 Lake sediments were lower by about 1000 $\mu S\ cm^{-1}$; Schumacher 1 Lake was lower by about 600 $\mu S\ cm^{-1}$, and at Little Pearl Lake the sediments were over 2000 $\mu S\ cm^{-1}$ lower. The remainder of the locations had sediments within the same range ± an error of 10 %.

Chara vulgaris standing biomass varied considerably at different locations. While the nickel tailings pond (Langmuir) had the highest standing crop, Irrigation Lake and Middle Triple Lake had the lowest. The diversity in the physical and biological habitats was large and the measures of standing biomass and oospores density were too dynamic to relate them to presence or absence of the algae. E_h, sediment quality, conductivity, and water depth also varied considerably at each location, but not with standing crop.

Standing biomass was determined in October 2001 and again in April 2002 for the population in Little Pearl Lake, Schumacher 1, Middle Triple, Irrigation Lake, the latter two were uncontaminated. In Little Pearl Lake, biomass dropped from 145 to 67 g(dw) m^{-2}, suggesting that the *C.vulgaris* did not overwinter well in that deep lake. However, in Schumacher 1, with a depth half that of Little Pearl lake, standing biomass doubled over the same period. Unfortunately in the adjacent pond Schumacher 2 growth was not monitored over the winter. These oospore observations may suggest that populations grow better in shallower lakes, which have higher oospore counts than deeper lakes. In fact, a linear correlation between depth and oospore counts is significant at 0.05 % level. However systematic field observations are needed to follow up on these random observations.

Table 2: Sediment characteristics oospores and standing biomass								
Site	**Texture**	**LOI**	**pH**	**E_h**	**Cond.**	**Oospore density**	**Standing Biomass**	**Standing Biomass**
		%		**mV**	**$\mu S\ cm^{-1}$**	**$N\ m^{-2}$ x 10^2**	**gdw m^{-2} 01/15/2002**	**gdw m^{-2} 04/22/2002**
Northern Saskatchewan (58o 11' N, 103° 41' W)								
Link lake lower 1988	Organic					n.a.	150	
Upper Link lake	n.d.						350	
Timmins, Northern Ontario (48° 35' N, 81 ° 52 ' W)								
Hollinger 1985	tailings	n.d.	8	n.d	190	n.a.	760	
Irrigation Lake	Organic	26	7.1	-24	172	0	Very low	Very low
Langmuir Nickel 1985	Tailings		8.3	n.a.	350	n.a.	2400	
Langmuir Nickel 2002	Tailings and organics	11	7.1	-72	684	1350	48	n.d.
Middle Triple Lake	coarse sand	1	7.1	-20	247	511	Very low	Very low
Little Pearl Lake	very fine organics	12	7.2	-116	970	57.2	145	67
Pamour 1985	Tailings	n.d.	8.2	n.d	230	n.a.	203	
Schumacher 1	organics	12	6.8	-31	596	529	38	77
Schumacher 2	organic	5	7.3	-99	1227	1580	88	n.d.
Southwestern Ontario (42° 22' N, 82° 22' W)								
St. Clair Lake	silt	8	6.8	-31	596	529	21	n.d.

n.d. = not determined n.a. = not available
very low = present but too sparse to collect

3.4 Water/biomass elemental content

While no correlations within the elemental concentrations in the water were statistically significant, the concentration factors (biomass to water ratio of an element) indicated strong enrichment of elements – especially with those elements comprising the first and second factors of the biomass correlation matrix. The biomass seemed to concentrate these elements regardless of their absolute concentrations in the water. The analysis of concentration factors provided a strong hint that the alkali earth elements were the drivers of the elemental composition of *C. vulgaris* biomass. This is not surprising from an ecological perspective, given the calcification of the *C. vulgaris* cell wall.

Elemental concentrations in *C. vulgaris* and *N. flexilis,* as compared to the water in which they live, varied considerably. A relatively large error has to be associated with the absolute concentration values given the uncertainty contributions from field sampling, sample preparation and analysis, and the change in the analytical precision with which elemental concentrations were generated by various methods (AAS, ICP-OES, ICP-MS), not to mention the change in technology over the last two decades. We therefore present the mean algal and water concentrations on a logarithmic scale. The data are presented in four groups, 1) the control or the uncontaminated lakes, 2) all gold mines and 3) other mines, including coal, Ni and Zn. The 4^{th} group is a single location of *N. flexilis* growing uranium mill effluent. The elements are presented according to their chemical classification in the periodic table reflecting somewhat similar chemical characteristics. Thus, the element groups are alkali metals (K, Na), alkali earth metals (Ba, Ca, Mg, Sr) transition metals (Cu, Fe, Mn, Ni, and Zn), post transition metals (Al), metalloids (As, B), other non-metals (Se, P, S), and actinoids (U).

In Figure 2a, the concentrations K and Na are presented, with both elements present in water at about the same concentration, at or slightly below 10 mg L^{-1}. These elements were concentrated in the biomass by up to 2 orders of magnitude. In all groups the concentrations are remarkably consistent between 7000 and 12000 mg kg^{-1}.

Figure 2a: The concentration of K and Na in water and *Chara vulgaris* and *Nitella flexilis* biomass.

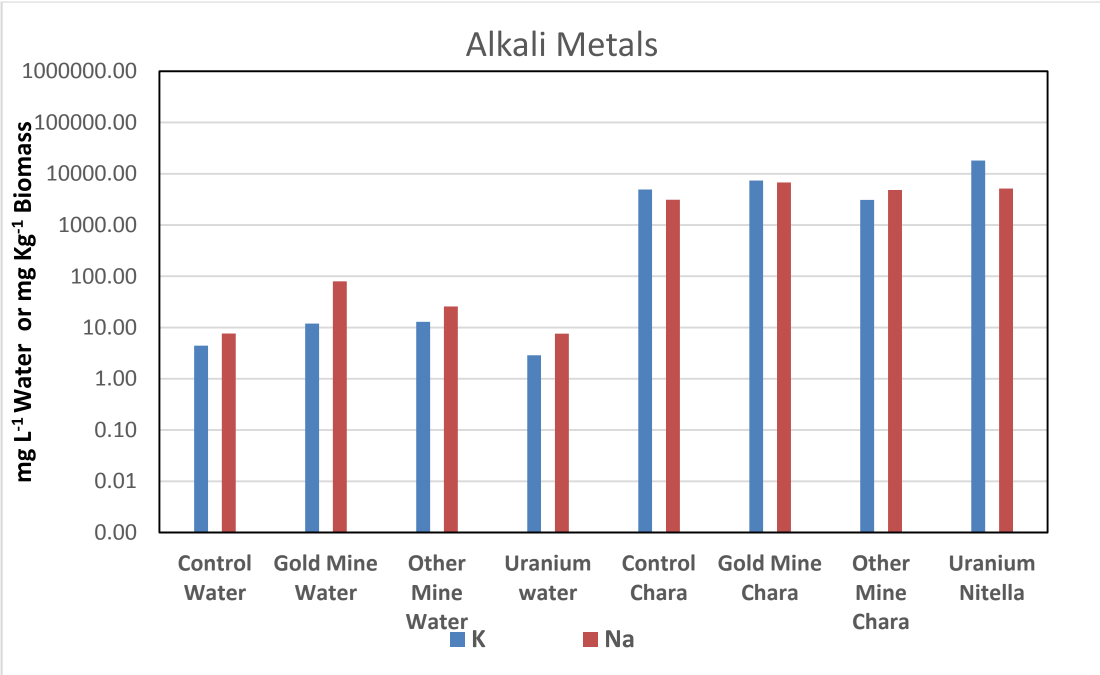
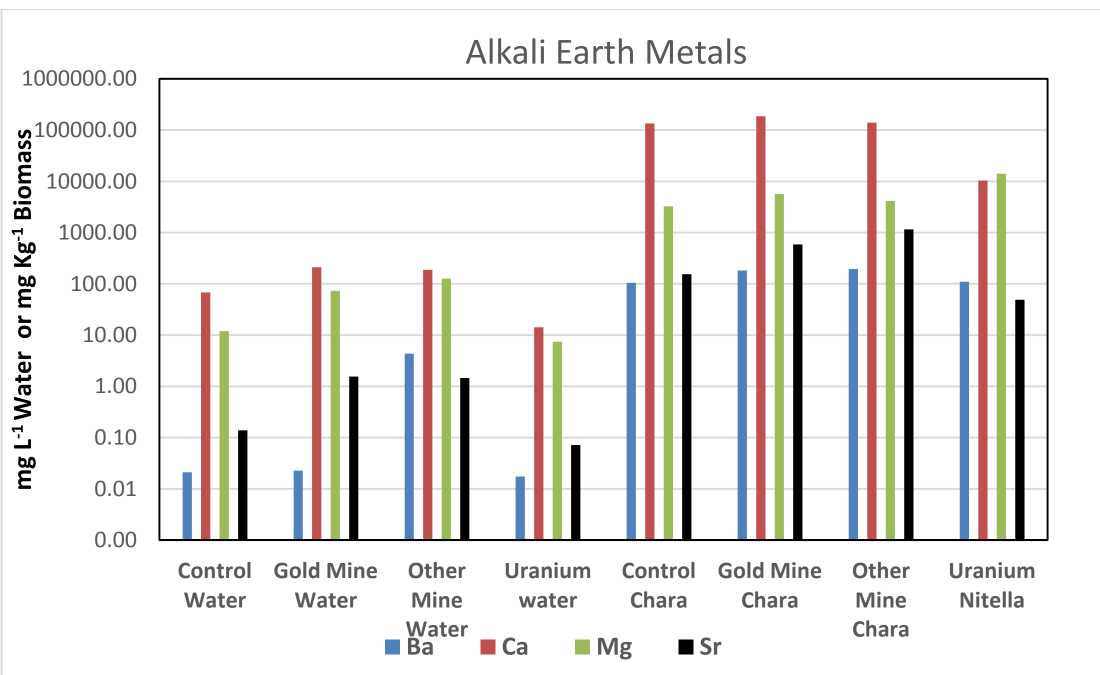
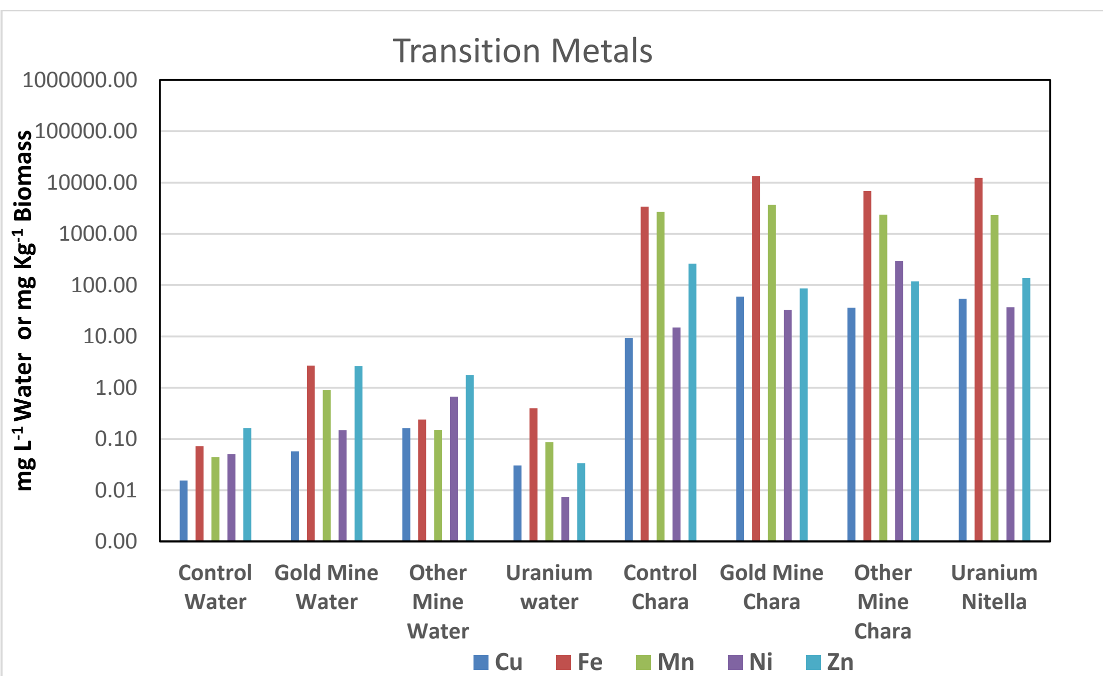


Figure 2b: The concentration of Ba, Ca, Mg, and Sr in water and *Chara vulgaris* and *Nitella flexilis* biomass.



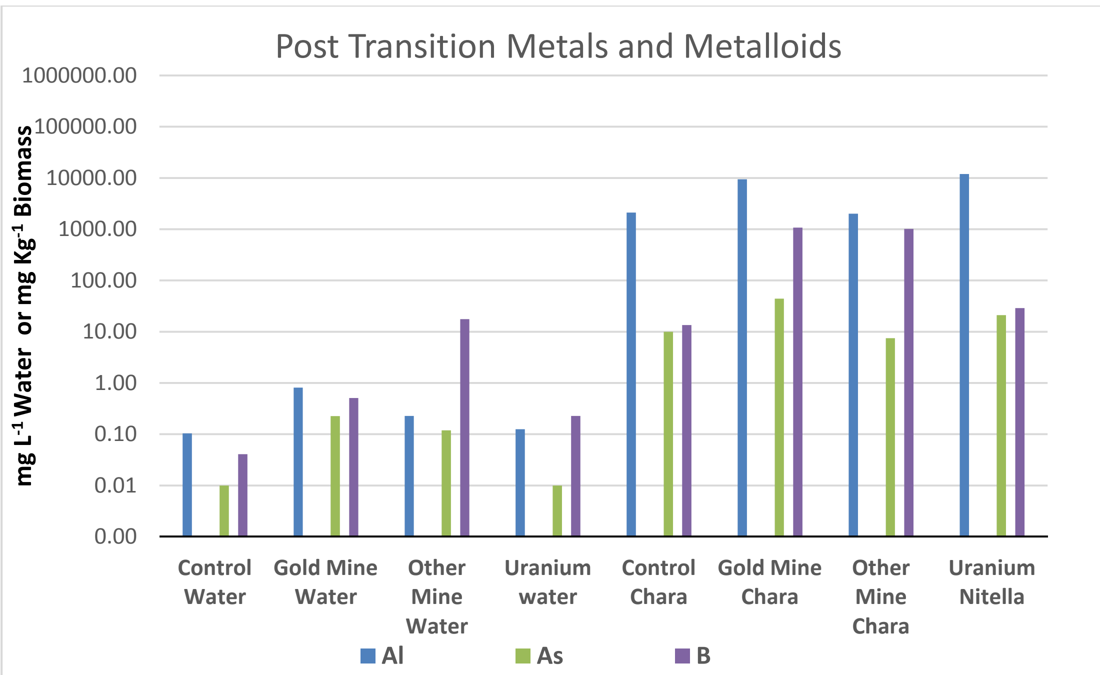
Both the alkaline earth and transition metals are physiologically relevant drivers of growth, as inferred from the extensive study of calcification of charophytes in fresh and brackish water. The enrichment factors for all the alkaline metals (Figure 2b) are 3 to 4 orders of magnitude (OM) in the biomass, regardless of water concentration (10-100 mg L^{-1} for Mg and Ca, and 0.01 to 1.0 mg L^{-1} for Ba and Sr). The distribution of the transition metals in Figure 2c displays an interesting pattern. The low concentration of the transition metals in the waters of the control lakes and the uranium mines reflects a 4 OM enrichment of Fe and Mn similar to Ca and Mg, whereas Cu, Ni and Zn are increased only by 3 OM. It suggests that these elements are co–precipitated with the carbonates.

Figure 2c: The concentration of transition metals in water and *Chara vulgaris* and *Nitella flexilis* biomass.



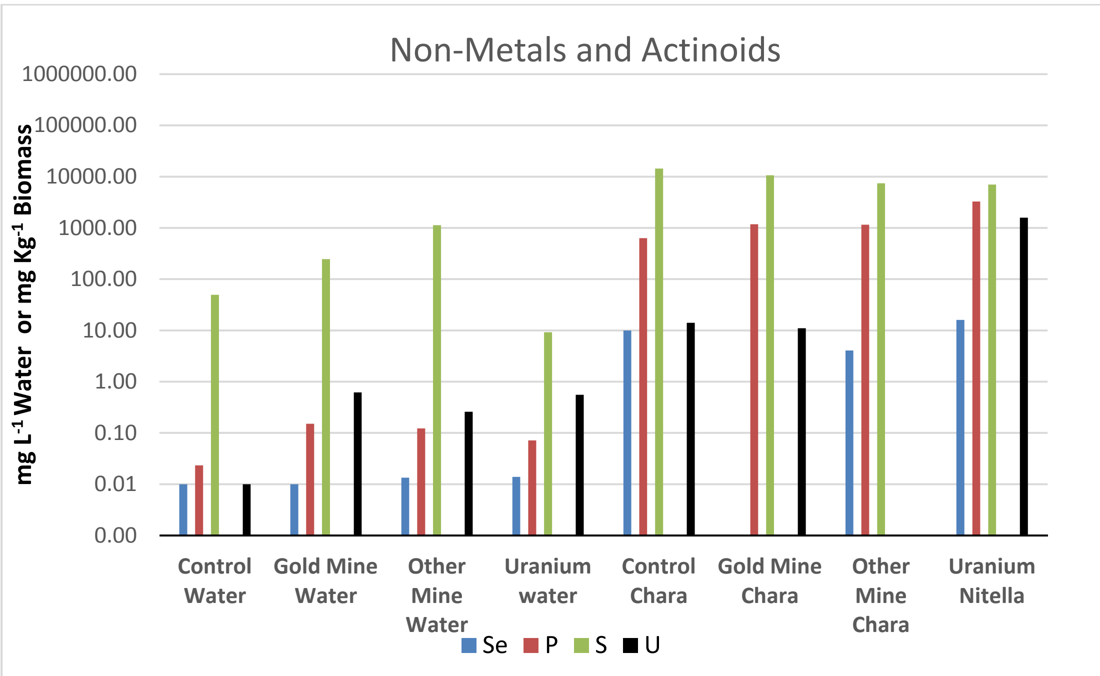
The post-transitional metals and metalloids (Figure 2d) have enrichment factors from 3 to 4 OM, which is similar to the carbonates. These elements often form complexes and or occur as hydroxides and may adsorbed onto the carbonate-encrusted cell wall.

Figure 2d: The concentration of post transition metals and metalloids in water and *Chara vulgaris* and *Nitella flexilis* biomass.



The last group of non-metals and actinoids Se, P, S and U display a less uniform pattern of enrichment compared to the other elemental groups (Figure 2e). Sulphur was enriched in the biomass one or two OM and phosphate nearly 4 orders higher in the biomass. Selenium is enriched by nearly 3 OM, while uranium enrichment factors are 2 OM in uncontaminated water, 1 OM for the gold mine biomass and 3 OM for the *N. flexilis* in the uranium biomass. These elements, although in the same chemical group, do not behave in a similar manner.

Figure 2e: The concentration of non-metals and actinoids in water and *Chara vulgaris and Nitella flexilis* biomass.



Uranium forms complexes with phosphate. We expected that both phosphate and uranium would be enriched in the biomass similarly. This only held for the uncontaminated lakes (control group) with increases of 3 OM for both elements. In the gold mine biomass, the increase in uranium was only 1 OM, whereas phosphate increased 4 OM. For the base metal, coal and uranium mines, the concentration of phosphate increased 4 times. This group of elements forms species with other elements in the water (speciation). These compounds would not be similarly affected as the other groups of elements, hence the irregular accumulation.

The sulphur concentration in the all waters ranged from 10 to 1000 mg L^{-1}, but the sulphur concentrations in the biomass of all groups were around 10,000 mg kg^{-1}. No other element reflected such a broad concentration range in the water with a nearly constant concentration in the biomass. It does suggest that sulphur may is an essential element the *Chara vulgaris* and *Nitella flexilis* biomass.

4. Discussion

It is well known that charophytes vary widely in their breadth of ecological tolerance. Whereas some species have very specific habitat requirements others are widespread and cosmopolitan with a broad ecological amplitude (Corillion, 1975; Mann *et al*., 1999). *Chara vulgaris* is widespread throughout North America (Wood, 1965) and Europe (Krause, 1997) and is either considered to be, part of a cosmopolitan species which includes the widespread species *C. contraria* (Wood 1965; Moore 1986) or is closely related to the latter (Grant and Proctor, 1972; Krause 1997; Schubert and Blindow 2004). In North America, *C. contraria* undoubtedly has the widest ecological tolerance of any *Chara* species (personal observation, R. Scribailo). *Nitella flexilis* is the most widespread member of the genus in North America (Allen 1892) and Europe (Krause 1997; Schubert and Blindow 2004) and is found in a wide variety of habitats from shallow wetlands to deep lakes of varying trophic status. Given the wide ecological amplitude demonstrated by both *C. vulgaris* and *N. flexilis* it is not surprising that they are found naturally occurring in mining waste streams and represent good candidates for research into factors that promote their growth under these circumstances.

We have collected *Chara vulgaris* from a number of mining site effluent ponds and ditches, as well as natural ponds and lakes. During these investigations we noted that the *C. vulgaris* was present in some water bodies, but not others that had similar characteristics. To see if we could determine any causal effects related to environmental parameters, we took our entire environmental data set, including field measurements, lab elemental data, and submitted them to a blind statistical analysis. To address the results of the statistical tests, we needed to think about elements (nutritional or pollutant) in a geochemical context. In other words, we had to look at how a biological entity (*Chara*) interacts with its aqueous environment on a geochemical level.

The multivariate analysis of the elemental concentrations for waters, sediments and biomass revealed that only the biomass data yielded significant correlations across sites. This result suggests that the concentration of elements in the biomass is largely independent of the concentration in the water. *Nitella flexilis* biomass originating from one lake system, for example, did not exhibit a significantly different elemental composition from the *C. vulgaris* sites even though it grew in water with markedly lower element concentrations. The absence of elemental correlations in the water and sediment data is somewhat expected given the fact that the mining ponds analyzed in this study span an area separated by thousands of kilometers in different climatic and geological settings with widely varying effluent compositions. This makes the correlations of the elements in the *C. vulgaris* biomass data even significant as the data collection covers a wide geographical area, vastly differing contaminated water and two genera of the charophytes.

Factor analysis indicates that these significant correlations are primarily driven by concentration similarities of three groups of elements. The first factor is composed of main group metals, especially the alkaline earth elements, while the second factor is a product of similarities in the composition of transition metals. The significance of these findings is uncertain and a further understanding would require controlled uptake experiments under laboratory conditions utilizing different combinations of elements.

Charophytes have been studied extensively as a means to remove metals from ponds and streams. Sooksawat *et al*. (2013) studied both *Chara aculeata* and *Nitella opaca* in the laboratory, feeding Cd, Pb and Zn to the algae. Zinc concentrations in *C. aculeata* reached 6700 mg kg^{-1}, while for *N. opaca*, the concentration dropped to around 1500 mg kg^{-1}. These are higher bioconcentration factors than are shown by our plants by about a factor of 10. Their data were also considerably higher than those found by Xing *et al*. (2013), who analyzed metal accumulations in non-characean macrophytes growing in the Yangtze River drainage basin. They reported strong positive correlations between concentrations of heavy metals in tissues of submerged macrophytes, probably because of co-accumulation of heavy metals. However, for most heavy metals, no significant correlations were found between submerged macrophytes and their surroundings. Interestingly, the concentrations of Li, Mg, Na and Sr in tissues of these submerged macrophytes significantly correlated with their corresponding water values, but not sediment values (Xing *et al*. 2013a).

Lambert and Davy (2010) looked at the presence/absence of charophytes against a number of environmental variables. They suggested that charophyte distribution was influenced most by nitrate and phosphate concentrations. This is to be expected, in that these are the primary growth nutrients for all plants. However, the next set of elements was more interesting. They showed that Cu, Cd, B, Co, Ni, and Mn were then the most influential, in decreasing order. Cu, Cd, Co, Ni and Mn are transition metals, while B is a metalloid. Calcium did not seem to be as important for the distribution, although they suggested that it was more important for calcium carbonate encrusting species than for those that did not. In another study, Vardanyan and Ingole (2006) collected aquatic macrophytes including *Chara vulgaris* from Savan Lake in Armenia and determined the concentration of 14 elements in the biomass. From the data, they determined which elements had the highest affinity for *C. vulgaris*. They found Ca, Mg and Fe were the greatest, followed by Mn, Al, Ba, Ti. The next group included Zn, Cu. The least concentrated elements were Cr, Co, Ni, Pb and Cd. These groupings are consistent with the data presented here. In our case, however, Cr, Co, Pb, Cd, and Ti were all below detection limits and not considered in the evaluations. Biomass of *C. vulgaris* from Lake Savan contained up to 414,000 mg kg^{-1} Ca, which was 2-3 times that found in our *C. vulgaris* (Vardanyan and Ingole, 2006). Thalli also contained 15,000 mg kg^{-1} Fe, and 15,000 mg kg^{-1} Mg. These were 2-3 x higher for Mg, but similar to those iron concentrations we encountered. However, metal accumulations on the cell wall are chemical reactions of the living cell wall bathed in carbonate dominated water chemistry, and as such, should be relatively similar across species and locations. The difficulty with most studies of this sort presented above is that they analyze only a subset of the elements present in the water or biomass, and thus, may arrive at different affinities. How do we explain these varied affinities with elemental geochemistry?

Since our data showed no statistically sound linear relationship between water and biomass, we need another explanation. The alkali earth metals (K, Na) are indispensable in the control of plant turgor pressure, enzyme activation, and a host of other functions. So, it is not surprising that these are the most important elements. Along with these alkali elements, the alkaline earth elements, Ca, Mg, Sr, and Ba are, perhaps, uniquely important to the characeae, where they are important in biomineralization (Kawahata *et al*. 2013).

This unique relationship between Ca and other elements associated with the characean cell wall was described in the review by Malaviya and Singh (2012), in the context of the remediation of uranium in both soil and water. Quoting the work of Dakovic *et al*. (2008), they concluded that the removal of uranium was mainly a function of the cell wall characteristics of *Chara fragilis,* where co-precipitation of uranyl species occurs with $CaCO_3$, at pH values above 5. Their experiments showed that live *C. fragilis* formed different crystalline forms (aragonite and rutherfordine) on the cell walls. This was confirmed by both Fourier Transform Infrared (FTIR) spectrometry and X-ray diffraction. Although *C. vulgaris* is a different species, we assume that the transport and exchange systems are similar to those of *C. fragilis,* and thus the high concentrations of the alkaline earth elements in the biomass are likely, in part, crystalline and co-precipitated metals. Gomes and Asaeda (2010) studied the impact of Ca and Mg on the growth of *Nitella pseudoflabellata*. Growing *N. pseudoflabellata* in varying concentrations of Ca and Mg, they noted that Mg interacts with the process of Ca accumulation, by competing with the Ca for precipitation of calcite. The calcification of the thallus depends on both bicarbonate and calcium in the water.

Cell walls calcify in the alkaline regions of their surface. These $CaCO_3$ encrustations may either be formed by precipitation due to alkalization of the medium (Spear *et al.*, 1969), or involve active Ca^{2+} and H^+ ATPases (McConnaughey and Falk, 1991; Schmolzer *et al*. 2011). These encrustations are likely composed of the precursors of secondary minerals such as Smithonite (Zn) Azurite (Cu) Siderite (Fe), Witherite (Ba) soda ash (Na) and dolomite for Ca and Mg (Reitner and Thiel, 2011).

The transition metals, especially those on the first row of the periodic table (Cu, Fe, Mn, Ni, and Zn), are usually divalent, although some can be trivalent, depending on the redox state. Transition metals can be adsorbed onto cell wall protein moieties, such as amino and carboxl groups (Kalin *et al*. 2005). Since charophyte cell walls have both acidic and basic bands, both anions and cations can be bound to different areas on the cell wall.

Data on sulphur concentrations in water versus biomass were of interest from this study because of the relatively constant concentration in biomass despite widely varying levels in the surrounding water. It does suggest that sulfur may have a growth-stimulating effect and more than 10 mg L^{-1} are needed in the water for *Chara vulgaris,* but not for *Nitella flexilis*. Sulfide in soils has been shown to have a toxic effect on the growth of *N. flexilis* (Van der Welle *et al*. 2006) and this species is associated with habitats with low concentrations of sulphate (De Lyon and Roelofs 1986). As sulfur and sulphides are known to play a role in rhizoid formation (Anderson, 1958) the role of sediments may be essential with respect to population growth (Smith, 1987)

Charophytes are generally considered early successional species predominating in oligotrophic to mesotrophic lakes but typically declining in eutrophic conditions (e.g., Blindow 1992: Van der Berg *et al*., 1999). Bornette *et al*. (1996) noted that *Chara vulgaris* has substantial tolerance to nutrient enrichment and to variations in conductivity and alkalinity, all of which were observed to show wide variations across our study sites. Baastrap-Spohr *et al*. (2013) recently noted that both *C. vulgaris* and *N. flexilis* have both greatly increased in abundance over the last 70 years in Danish Lakes despite the decline of most species due to eutrophication. Simons and Nat (1996) reported both of these species to be the most abundant in the Netherlands despite similar downward declines in water quality. It would be interesting to address the role of sulphur as one of the mechanisms responsible for stress tolerance given that the biomass contains relatively constant suphur concentration as indicated by this data set, despite widely varying habitat conditions.

5. Conclusions

.

We asked specific questions in relation to the elemental composition of the biomass. 1) Does an analysis of the physical and chemical characteristics of mine waste water habitats, spread over a wide geographical area, reveal any ecologically-relevant similarities? The observations of this data set do not indicate any obvious similarities. 2) Are the elemental profiles of the water and the sediment indicative of concentrations found in biomass? Our data clearly suggests that this is not the case. 3) Are there significant correlations among the elements in the biomass which might suggest that they are of relevance to growth? While we did not include nitrogen or carbon in the analysis, our data suggested that P (as a non-metal) did not contribute any significant amount to our understanding of characean distribution. We did, however, find significance to Ca, Mg. These are definitely involved in characean growth. We also found that S may play a role in the growth and distribution of the charophytes studied. It would be interesting to address the role of sulphur as one of the mechanisms responsible for stress tolerance given that the biomass contains relatively constant sulphur concentrations, as indicated by this data set, despite widely-varying habitat conditions.

The multivariate analysis of the three data matrices holding elemental concentration of waters, sediments and biomass revealed that only the biomass data showed correlations that were significantly different from statistical noise. This result seems natural. There is no reason to see a connection between waters and sediments in lakes and mining ponds separated by hundreds of kilometers. In the case of the biomass data, there is only one single link namely the cell wall characteristics of *Chara vulgaris and Nitella flexilis*. Factor analysis indicates that only a few factors are responsible for this elemental composition with only three relevant factors. Two factors could be identified where the first factor holds main group metals, especially the alkaline earth elements. The importance of the statistical analysis of *C. vulgaris* biomass data rests on the fact that the methods are model-free. Hence, the only assumption going into this analysis is that the data represent an unsystematic selection from a homogeneous population. This population is the set of all *Chara*-growing sites in the respective regions. Thus, as this data set is a purely empirical set of observations, further questions can be raised in relation to bio-concentration factors, often used in phytoremediation of soils and water. Indeed it appears that the geochemistry of the elements in the water dominates the enrichment of the biomass with the respective elements. The presented simple chemical perspective of the biomass composition suggests that certainly minima or maxima of elemental concentrations should be defined with caution in relation to toxicity and uptake for both genera of charophytes.

The analysis of field data provided in this paper and obtained from over two decades of ecological engineering approaches to restore alkaline, metal-laden mine waste management areas indicates that *Chara vulgaris* and *Nitella flexilis* can remove significant quantities of metals from mining waste streams and ponds. The amount of metal removed depends on the geochemical characteristics of the metal.

6. References

Allen, S.E., Grimshaw, H.M., Parkinson, J.A., Quarmby C., 1974. Chemical analysis of ecologicial materials. Blackwell Scientific Publications, 557.

Allen T.F., 1892. The Characeae of America, Part II, Fascicle I. T. F. Allen, New York.

Anderson, R.G., 1958. The growth and reproduction of *Chara* in a definable nutrient medium. Ph.D. Thesis. University of Nebraska, Department of Botany.

Arancibia-Avila, P., Coleman, J.R., Russin, W.A., Graham, J.M., Graham, L.E., 2001. Carbonic anhydrase localization in charophycean green algae: Ecological and evolutionary significance. International Journal of Plant Science 162(1), 127-135.

Baastrup-Spohr, L., Iverson, L.L., Dahl-Nielsen, J., Sand-Jensen, K., 2013. Seventy years of changes in the abundance of Danish charophytes. Freshwater Biology 58, 1682-1693.

Blindow, I., 1992. Decline of charophytes during eutrophication: comparison with angiosperms. Freshwater Biology 28, 9-14.

Blindow, I., 2000. Distribution of charophytes along the Swedish coast in relation to salinity and eutrophication. International Rev. Hydrobiol. 85. (5-6) 707- 717

Blindow, S., H., 2004. Charophytes of the Baltic Sea. A.R.G. Gantner Verlag.

Bornette, G., Guerlesquin, M., Henry C.P., 1996. Are the Characeae able to indicate the origin of groundwater in former river channels? Vegetatio 125, 207-222.

Box, R.J., 1987. The uptake of nitrate and ammonium nitrogen in *Chara hispida* L.: The contribution of the rhizoid. Plant, Cell and Envrion. 10, 169-176.

Box, R.J., 1988. Preliminary comparison of dry weight increase with short term uptake of C, N and P in cultured *Chara hispida* plants. Biochemie und Physiology der Pflanzen 183 (6), 503-507.

Cattell, R.B., 1966. The Scree Test For The Number Of Factors. Multivariate Behavioral Research 1, 245-276.

Chakravarti, I.M., Laha, R.G., Roy, J., 1967. Handbook of Methods of Applied Statistics, Vol. I. Wiley & Sons New York, 392-394.

Clabeaux, B.L., Navarro, D.A.G., Aga, D.S., Bisson, M.A., 2011. Cd tolerance and accumulation in the aquatic macrophytes Chara australis: Potential use for charophytes in phytoremediation. Environmental Science and Technology 45(12), 5332-5338.

Cooley, W.W., Lohnes, P.R., 1971. Multivariate data analysis. J. Wiley and Sons Inc., New York, 339 p.

Coops, H., 2002. Ecology of charophytes: an introduction. Aquatic Botany 72, 205-208.

Corillion, R., 1975. Flores des Charophytes du massif Armoricain et des contrees voisines d'Europe occidentale. Jouve Editeurs, Paris.

Culbert, J., 1984. Surficial Uranium Deposits in Canada. IAEA-TecDoc - 332: Surficial Uranium Deposits, Report of the Working Group on Uranium Geology Organized by the International Atomic Energy Agency, 179–192.

Dakovic, M., Kovacevic, M., Andjus, P.R., Bacic, G., 2008. On the mechanism of uranium binding to cell wall of Chara fragilis. European Biophysics Journal 37, 1111-1117.

De Lyon M.J.H., Roelofs, J.G.M., 1988. Waterplanten in relatie tot waterkwaliteit en bodemgesteldheid deel 2: Tabellen. Laboratory of Aqautic Ecology, Katholieke University, Nijmegen, The Netherlands.

Freytet, P., Verrecchia, E.P., 2002. Lacustrine and palustrine carbonate petrography: an overview. J. Paleolimnology 27, 221-237

Gomes, P.I.A., Asaeda, T. 2010. Charophytes as a hyperaccumulator of heavy metals: role of calcification and applicability in restoration of polluted water bodies. Rep. Res. Edu. Ctr. Inlandwat. Environ. 6,73-81.

Golub, G.H., Reinsch, C., 1970. Singular value decomposition and least squares solutions. Numer. Math. 14, 403-420.

Grant, M.C., Proctor, V.W., 1972. *Chara vulgaris* and. *C. contraria*: Patterns of reproductive isolation for two cosmopolitan species complexes. Evolution 26, 267-281.

Kalin, M., Fyson, A., Wheeler, W.N., 2007. The chemistry of conventional and alternative treatment systems for the neutralization of acid mine drainage. Sci. Tot. Environ. 366, 395-408.

Kalin, M., Kießig, G., Küchler, A., 2002a., . In: Merkel, B., Planer-Friedrich, B., Wolkersdorfer, C. (Eds.), Ecological water treatment processes for underground uranium mine water: Progress after three years of operating a constructed wetlandUranium in the Aquatic Environment. Springer, Berlin Heidelberg, 587-596.

Kalin, M., Smith, M., Wittrup, M., 2002b. . In: Merkel, B., Planer-Friedrich, B., Wolkersdorfer, C. (Eds.), Ecosystem restoration incorporating minerotrophic ecology and Stoneworts that accumulate 226Ra Uranium in the Aquatic Environment. Springer, Berlin Heidelberg, 495-504.

Kalin, M., Wheeler, W.N., Meinrath, G., 2005. The removal of uranium from mining waste water using algal/microbial biomass. Journal of Environmental Radioactivity 78, 151-177.

Khanaqa, P.A., Karim, K.H., Thiel, V., 2012. Characeae-derived carbonate deposits in Lake Ganau, Kurdistan Region, Iraq. International Journal of Paleontology, Sedimentology and Geology DOI 10. 1007/s10347-012-0354-9.

Krause, W., 1997. Charales (Charophyceae). Süsswasserflora von Mitteleuropa. Band 18. Gustav-Fischer verlag, Jena.

Kufel, L., Kufel, I., 2001. Chara beds action as nutrient sinks in shallow lakes – a review. Aquat. Bot. 72, 249-260.

Lambert, S.J. and Davey A. 2010 Water Qualtiy as a threat to aquatic plants: discriminating between the effects of nitrate, phosphate, boron and heavy metals. New Phytologist 189, 1051-1059.

Malaviya, P., Singh, A., 2012. Phytoremediation Strategies for Remediation of Uranium-Contaminated Environments: A Review. Critical Reviews in Environmental Science and Technology 42, 2575-2647.

Mann, H., Proctor, V.W., Taylor, A.S., 1999. Towards a biogeography of North American charophytes. Australian Journal of Botany 47, 445-458.

Marquardt, R., Schubert, H., 2009. Photosynthetic characterization of *Chara vulgaris* in bioremediation ponds.

McConnaughey, T., 1998. Acid secretion, calcification, and photosynthetic carbon concentrating mechanisms. Can. J. Bot. 76, 1119-1126.

McConnaughey, T., Falk, R.H., 1991. Calcium-proton exchange during algal calcification. Biol. Bull., 180-185.

Moore, J.A., 1986. Charophytes of Great Britain and Ireland. Botanical Society of the British Isles.

Morrison, D.F., 1976. Multivariate Statistical Methods 2nd ed., McGraw-Hill, Düseldorf, F.R.G.

Pelechaty, M., Pukacz, A., Apolinarskai, K., Pelchata, A., Siepak, M., 2013. The significance of *Chara* vegetation in the precipitation of lacustrine calcium carbonate. Sedimentology 60, 1017-1035.

Prasad, M.N.V., Freitas, H.M.d.O., 2003. Metal hyperaccumulation in plants - Biodiversity prospecting for phytoremediation technology. Electronic Journal of Biotechnology 6, No. 3 December 15 2003.

Rand, M.C., Greenberg, A.E., Taras, M.J., 1976. Standard Methods for the Examination of Water and Wastewater. Fourteenth Edition. American Public Health Association, American Water Works Association and Water Pollution Control Federation, 1193.

Ray, S., Klenell, M., Choo, K.S., Pedersen, M., Snoeijs, P., 2003. Carbon acquisition mechanisms in *Chara tomentosa*. Aquatic Botany 76(2), 141-154.

Reitner, J., Thiel, V. Eds., 2011. The Encyclopedia of Geobiology. Springer Verlag, N.Y., 927.

Rodrigo, M.A., Rojo, C., Alvarez-Cobelas, M., Cirujano, S., 2007. *Chara hispida* beds as a sink of nitrogen: Evidence from growth, nitrogen uptake and decomposition. Aquatic Botany 87, 7-14.

Scheffer, M., 1999. The effect of aquatic vegetation on turbidity; how important are the filter feeders? Hydrobiologia 408/409, 307-316.

Simons, J., Nat, E., 1996. Past and present distribution of stoneworts (Characeae) in the Netherlands. Hydrobiologia 340, 127-135.

Smith, M.P., 1987. Phosphorus Nutrition in *Chara vulgaris*. M.Sc. Thesis University of Toronto, Department of Botany.

Schmölzer, P.M., Höftberger, M., Foissner, I., 2011. Plasma membrane domains participate in pH banding of *Chara* intermodal cells. Plant Cell Physiology 52, 1274-1288.

Schubert, H., Blindow, I., (Eds.)., 2004. *Charophytes of the Baltic Sea* (No. 19). Gantner.

Snedecor, G.W., Cochrann, W.G., 1989. Statistical Methods, 8th ed. Iowa State University Press Ames/USA.

Sooksawat, N., Meetam, M., Kruatrachue, M., Pokethitiyook, P., Nathalang, K., 2013. Phytoremediation potential of charophytes: Bioaccumulation and toxicity studies of cadmium, lead and zinc. Journal of Environmental Sciences 25, 596-604.

Spear, D.G., Barr, J.K., Barr, C.E., 1969. Localization of hydrogen ion and chloride ion fluxes in *Nitella.* J. Gen. Physiology 54, 397-414.

Van der Berg, M.S., Scheffer, M., Van Nes, E., Coops, H., 1999. Dynamics and stability of *Chara sp*. and *Potamogeton pectinatus* in a shallow lake changing in eutrophication level. Hydrobiologia 408, 335-342.

Van der Welle, M.E.H., Cuppens, M., Lamers, L.P.M., Roelofs, J.G.M., 2006. Detoxifying toxicants: Interactions between sulfide and iron toxicity in freshwater wetlands. Environmental Toxicology and Chemistry 25, 1592-1597.

Vardanyan, L.G., Ingole, B.S., 2006. Studies on heavy metal accumulation in aquatic macrophytes from Sevan (Armenia) and Carambolim (India) lake systems. Environ. Internat. 2, 208-218.

Warren, L.A., Haak, E.A., 2001. Biogeochemical controls on metal behavior in freshwater environments. Earth-Science Reviews 54, 261-320.

Wood, R.D., 1965. Monograph of the Characeae. In R.D. Wood and K. Imahori. A Revision of the Characeae. Vol. 1. Verlag von J. Cramer, Weinheim.

Xing, W., Wu, H., Hao, B. Huang W. Liu G., 2013a. Bioaccumulation of Heavy Metals by submerged Macrophytes: Looking for Hyperaccumulators in Eutrophic Lakes. Environmental Science & Technology 47, 4695-4703.

Xing, W., Wu, H., Hao, B., Liu, G., 2013b. Metal accumulation by submerged macrophytes in eutrophic lakes at the watershed scale. Environmental Science Pollution Research, DOI 10.1007/s11356-013-1854-z.

7.0 Acknowledgements

We thank Martin P. Smith who carried out the field work between 1980 and 2002. His dedication to the "Chara process " contributed to the success of many projects utilizing these algae to clean mine waste water in Ecological Engineering projects. The inclusion of the biomass growing in coal mine ponds was made possible due to the support of Prof. R. Warner and Dr. J. Unrine of the University of Kentucky with P.O. number 7500011726 under the ARIES (Appalachian Research Initiative for Environmental Science) program.

APPENDIX 1 : Raw data

Graphics sorting high to low concentrations

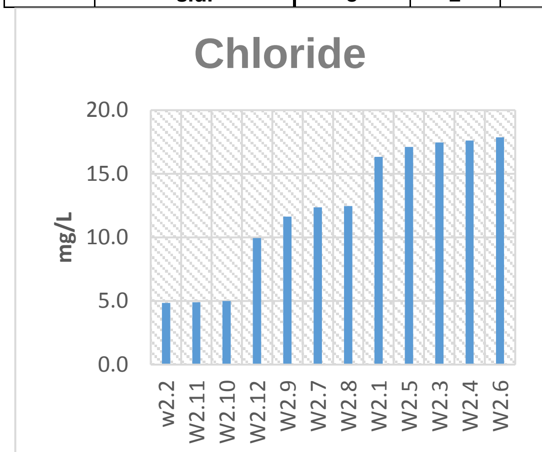
Table of contents

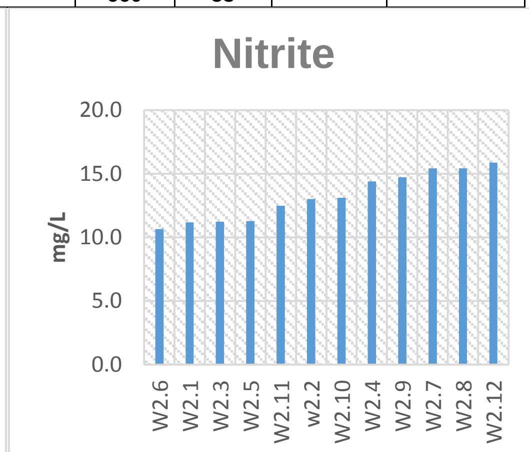
Note: The elemental composition of the biotic component of the vegetation and debris has been collected in part for also for future investigation to assess elemental cycling and ecological behavior of the ponds. A full interpretation of these data would be carried out during a field pilot study of the proposed treatment option.

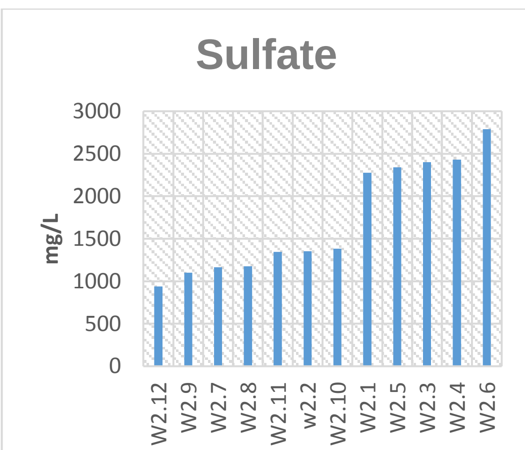
1. Concentrations of Cl, NO_2, F, SO_4, NO_3, Br,

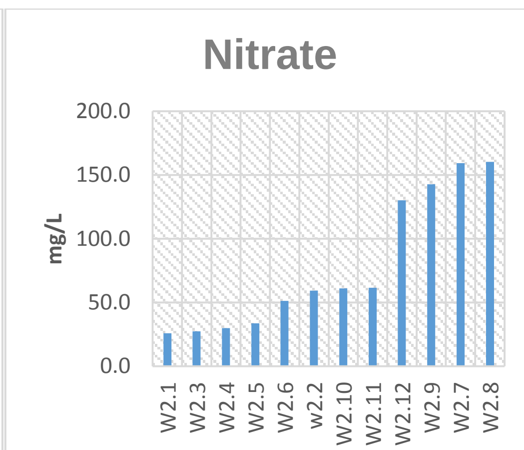
mg/L

		Chloride	Nitrite	Flouride	Sulfate	Nitrate	Bromide	Phosphate
b	BC #2 outflow	16.3	11.2	BDL	2275	25.8	BDL	BDL
w2.2	BC 1 outflow	4.8	13.0	BDL	1352	59.4	BDL	BDL
W2.3	BC 2	17.5	11.2	BDL	2401	27.3	BDL	BDL
W2.4	BC 2 inflow	17.6	14.4	BDL	2433	29.8	BDL	BDL
W2.5	BC 3	17.1	11.3	BDL	2341	33.8	BDL	BDL
W2.6	BC 3 inflow	17.9	10.7	BDL	2789	51.2	BDL	BDL
W2.7	Cliff Pond #28	12.4	15.4	BDL	1164	159.3	BDL	BDL
W2.8	Cliff Pond #28 rep	12.5	15.4	BDL	1174	160.2	BDL	BDL
W2.9	cliff pond outflow	11.6	14.7	BDL	1101	142.6	BDL	BDL
W2.10	H20 BC 1 ASC	5.0	13.1	BDL	1383	61.0	BDL	BDL
W2.11	H20 BC1 BSC	4.9	12.5	BDL	1346	61.5	BDL	BDL
W2.12	inflow cliffs	10.0	15.9	BDL	939	130.1	BDL	BDL
	average	**12.3**	**13.2**		**1724.8**	**78.5**		
	s.d.	**5**	**2**		**660**	**53**		









2. Unfiltered and 0.2 um filtered water

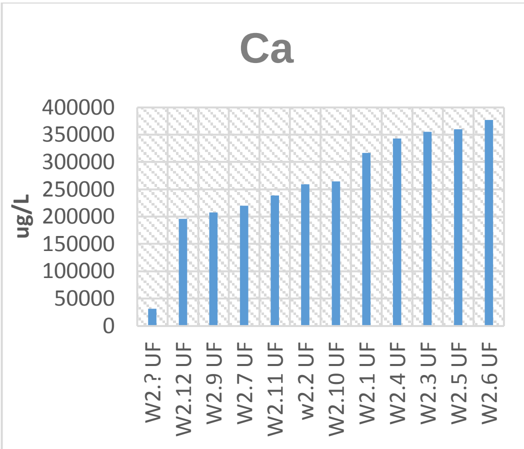
Unfiltered Total Recoverable						
Code	Location	**Na**	**Mg**	**K**	**Ca**	**Sr**
		ug/L	ug/L	ug/L	ug/L	ug/L
	M. DET. Lmt.	**0.8273**	**1.994**	**5.367**	**1.146**	**0.148**
W2.11 UF	H2O BC 1 BSC	7985	159800	20240	238800	2753
W2.10 UF	H2O BC 1 ASC	9972	177700	21940	264700	3032
W2.3 UF	BC# 2	15570	409900	34800	355500	3923
W2.5 UF	BC# 3	14200	383600	32940	359800	3856
W2.4 UF	BC# 2 Inflow	14730	389800	32770	343100	3807
W2.6 UF	BC# 3 Inflow	16400	447200	35390	376800	4912
w2.2 UF	BC 1 Outflow	10240	175400	21270	259200	3011
W2.1 UF	BC 2 Outflow	14740	381600	32330	316700	3453
W2.12 UF	Inflow Cliffs	9746	196500	18630	196000	532
W2.9 UF	Cliff pond Outflow	10540	215600	19410	207500	572
W2.7 UF	Cliff pond # 28	10950	218000	20310	220000	617
W2.? UF	Nist water	21350	8643	1665	31240	319
	Average	**12279**	**286827**	**26366**	**285282**	**2770**
	s.d	**2742**	**107976**	**6744**	**63773**	**1454**

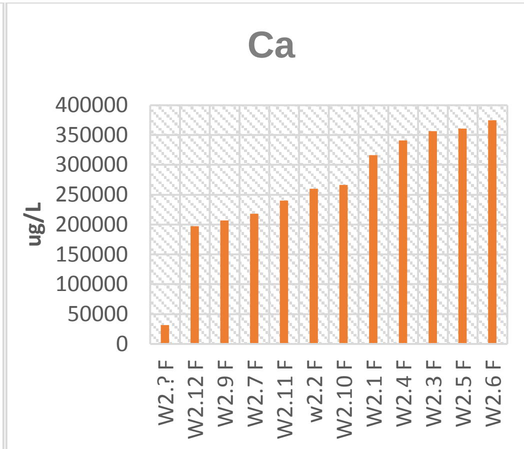
	Filtered 0.2um	**Na**	**Mg**	**K**	**Ca**	**Sr**
W2.11 F	H2O BC 1 BSC	7921	157400	20510	240200	2755
W2.10 F	H2O BC 1 ASC	9613	175200	22270	266500	3059
W2.3 F	BC# 2	15520	402400	34930	356300	3910
W2.5 F	BC# 3	14080	375000	32940	360600	3866
W2.4 F	BC# 2 Inflow	14540	380800	33040	341000	3783
W2.6 F	BC# 3 Inflow	16210	444100	36190	374700	4954
w2.2 F	BC 1 Outflow	9947	173800	21790	260100	3059
W2.1 F	BC 2 Outflow	14640	380200	33060	316500	3425
W2.12 F	Inflow Cliffs	9675	194400	19140	197000	537
W2.9 F	Cliff pond Outflow	10380	212300	19620	206700	572
W2.7 F	Cliff pond # 28	10910	215300	20810	218300	624
W2.? F	Nist water	21330	8675	2156	31620	331

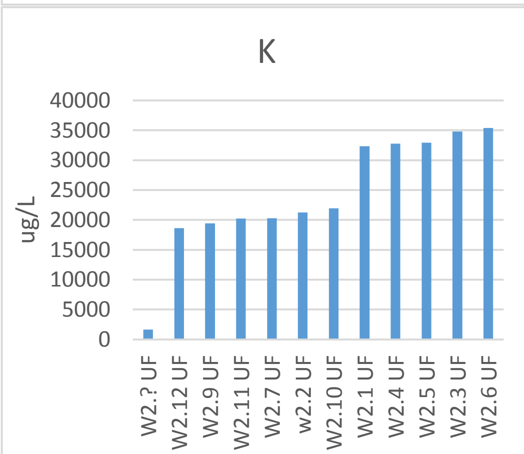
	Average	**12131**	**282809**	**26755**	**285264**	**2777**
	s.d	**2754**	**106361**	**6751**	**63436**	**1456**

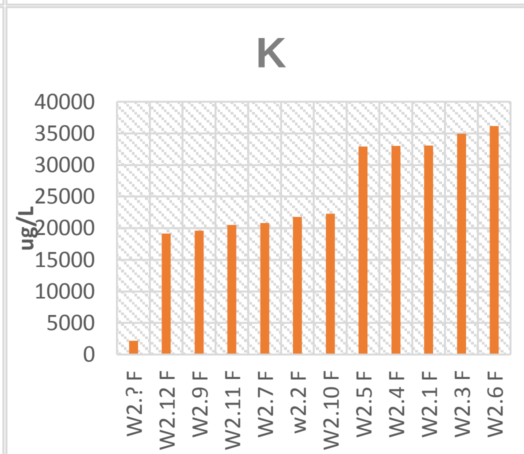
Method- ICP-MS Total recoverable by direct injection

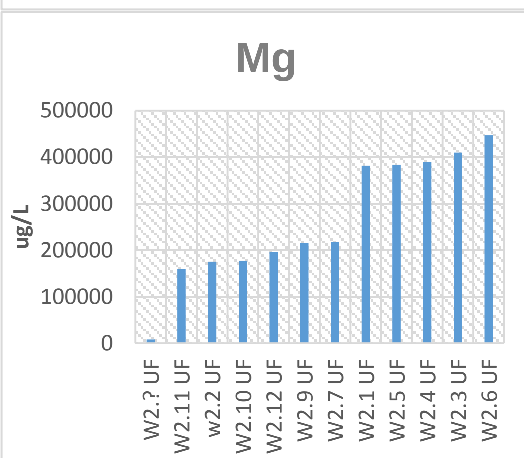
Unfiltered or filterd to 0.2 um

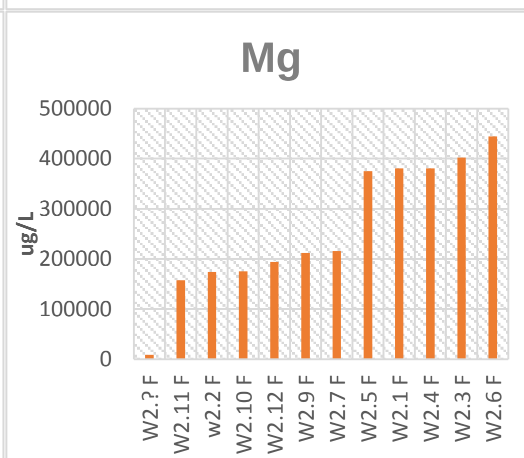




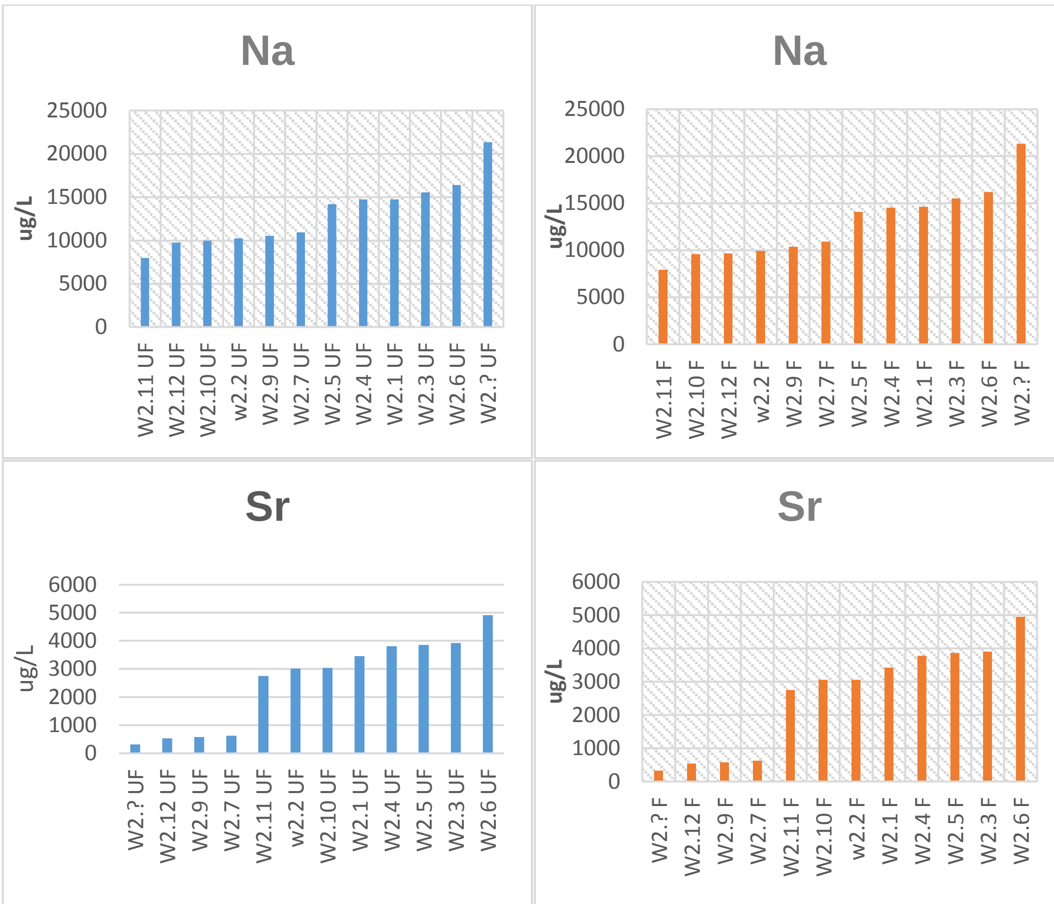








Blue: Filtered, Orange: Unfiltered



Blue: Filtered, Orange: Unfiltered

3. Conc. ug/Lof B, V, Cr, Mn , Fe, Co, Ni, Cu, Zn, As, Se

	ug/L	B	V	Cr	Mn	Fe	Co
w2.2	BC 1 Outflow	32.80	22.50	0.15	56.20	15.27	0.90
W2.1	BC 2 Outflow	70.65	38.35	0.09	17.80	6.98	0.40
W2.3	BC# 2	71.45	38.00	0.10	0.40	72.90	0.40
W2.4	BC# 2 Inflow	71.20	38.90	0.90	34.20	12.98	0.50
W2.5	BC# 3	70.65	36.30	0.10	48.20	18.92	0.50
W2.6	BC# 3 Inflow	74.25	44.15	0.09	2.20	0.25	0.40
W2.7	Cliff pond # 28	9.35	18.50	0.09	0.10	0.61	0.40

	ug/L	B	V	Cr	Mn	Fe	Co
W2.8	Cliff pond Outflow	9.50	19.70	0.10	0.20	8.64	0.40
W2.10	H2O BC 1 ASC	32.95	21.80	0.25	56.10	18.09	1.00
W2.11	H2O BC 1 BSC	31.00	20.90	0.25	1.00	15.67	0.40
W2.12	Inflow Cliffs	10.55	18.70	0.10	1.70	10.44	0.40
w2.2 F	BC 1 Outflow - filtered	42.15	23.25	**0.09**	**0.01**	**0.25**	0.40
W2.1 F	BC 2 Outflow - filtered	68.70	39.15	**0.09**	0.30	**0.25**	0.30
W2.3 F	BC# 2 - filtered	68.45	38.90	**0.09**	0.10	1.35	0.40
W2.4 F	BC# 2 Inflow - filtered	68.80	39.90	**0.09**	0.30	**0.25**	0.40
W2.5 F	BC# 3 - filtered	66.90	37.00	**0.09**	**0.01**	**0.25**	0.40
W2.6 F	BC# 3 Inflow - filtered	78.70	50.90	**0.09**	0.10	**0.25**	0.50
W2.7 F	Cliff pond # 28 - filtered	12.30	19.30	**0.09**	**0.01**	**0.25**	0.30
W2.9 F	Cliff pond Outflow - filtered	9.50	20.40	**0.09**	0.30	**0.25**	0.30
W2.00 F	Dupe 1-Cliff pond # 28	9.60	18.75	**0.09**	0.10	0.58	0.40
W2.11 F	H2O BC 1 ASC - filtered	33.80	21.55	**0.09**	0.20	0.32	0.40
W2.10 F	H2O BC 1 BSC - filtered	30.60	21.80	**0.09**	**0.01**	**0.25**	0.40
W2.12 F	Inflow Cliffs - filtered	10.55	18.05	**0.09**	**0.01**	**0.25**	0.30
W2.? F	Nist water	159.35	22.90	20.75	35.60	119.36	27.30
Wcert	Certified Value	154.00	36.93	19.90	38.02	98.10	26.40
	MDL	1.05	0.09	0.09	0.01	0.25	0.09
	Average	**51.91**	**29.06**	**1.76**	**11.73**	**16.11**	**2.56**
	s.d	**40**	**10**	**5**	**19**	**31**	**7**

Method -ICP-MS analysis -total recoverable by direct injection

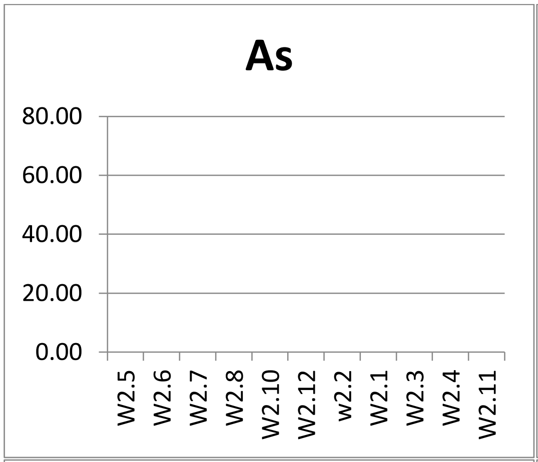
Unfiltered or filtered to 0.2 um

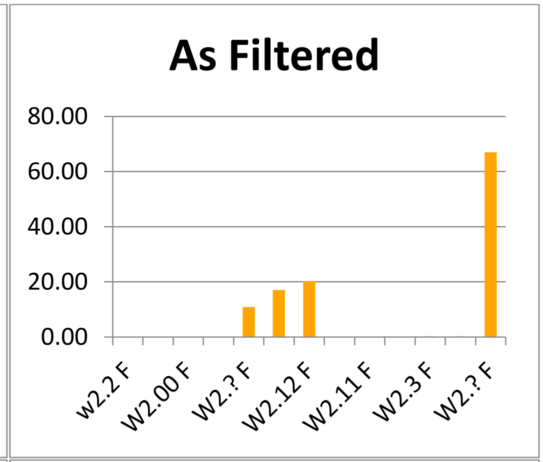
Nist Water and Certified values excluded from Average and Standard Deviation, yellow detection limit value

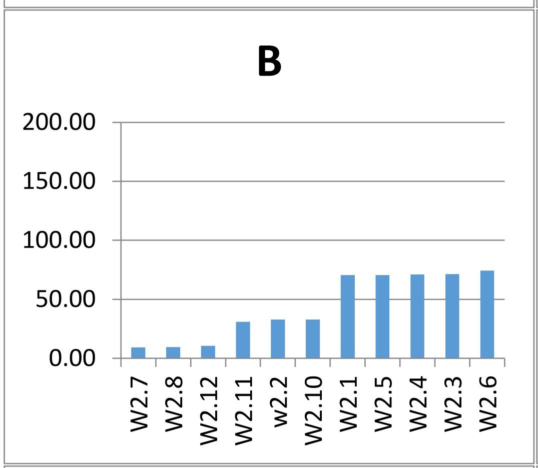
	ug/L	Ni	Cu	Zn	As	Se
w2.2	BC 1 Outflow	11.25	0.60	12.65	0.20	25.45
W2.1	BC 2 Outflow	2.20	0.10	5.15	0.20	10.20
W2.3	BC# 2	2.00	0.60	7.65	0.20	11.00
W2.4	BC# 2 Inflow	3.00	0.40	7.00	0.20	10.95
W2.5	BC# 3	3.05	0.40	7.90	0.15	11.40
W2.6	BC# 3 Inflow	4.15	0.30	6.55	0.15	17.10
W2.7	Cliff pond # 28	1.95	0.70	4.95	0.15	23.00
W2.8	Cliff pond Outflow	1.35	0.40	5.25	0.15	22.50
W2.10	H2O BC 1 ASC	11.80	0.70	18.20	0.15	26.75
W2.11	H2O BC 1 BSC	10.60	0.50	10.25	0.20	23.65
W2.12	Inflow Cliffs	2.00	0.40	6.05	0.15	22.20
w2.2 F	BC 1 Outflow - filtered	7.90	0.40	7.20	0.10	24.75
W2.1 F	BC 2 Outflow - filtered	1.75	0.60	5.70	0.15	9.50
W2.3 F	BC# 2 - filtered	1.95	0.20	8.20	0.20	10.20
W2.4 F	BC# 2 Inflow - filtered	2.10	0.20	5.60	0.20	10.05
W2.5 F	BC# 3 - filtered	2.70	0.30	6.90	0.15	10.30
W2.6 F	BC# 3 Inflow - filtered	3.10	1.70	8.25	0.15	17.05
W2.7 F	Cliff pond # 28 - filtered	1.75	0.30	4.55	0.10	21.95
W2.9 F	Cliff pond Outflow - filtered	1.45	0.50	5.40	0.15	21.75
W2.00 F	Dupe 1-Cliff pond # 28	1.80	0.10	5.20	0.10	21.40
W2.11 F	H2O BC 1 ASC - filtered	9.00	1.40	13.55	0.15	24.15
W2.10 F	H2O BC 1 BSC - filtered	8.80	0.40	8.95	0.15	24.10
W2.12 F	Inflow Cliffs - filtered	1.30	0.40	4.80	0.10	20.30
W2.? F	Nist water	71.10	28.00	96.50	66.95	10.90
Wcert	Certified Value	60.89	26.40	76.50	58.98	11.68
	MDL	0.09	0.01	0.23	0.09	0.23
	Average	**9.16**	**2.64**	**13.96**	**5.18**	**17.69**

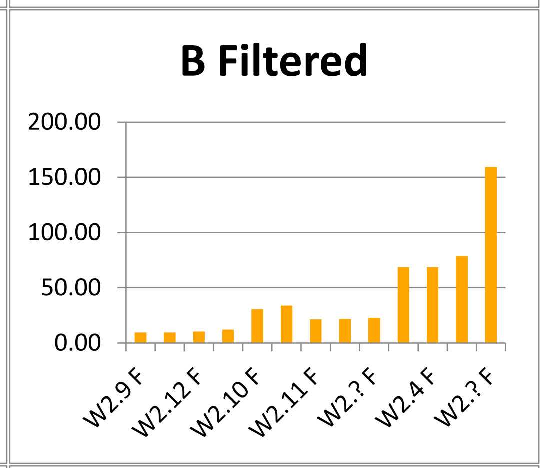
	ug/L	Ni	Cu	Zn	As	Se
	s.d	**17**	**7**	**22**	**17**	**6**

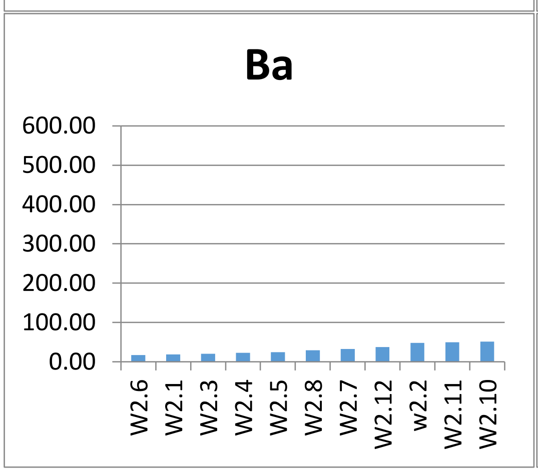
	ug/L	**Sr**	**Mo**	**Cd**	**Ba**	**Pb**
w2.2	BC 1 Outflow	3006.50	**2.89**	**0.31**	48.00	**0.09**
W2.1	BC 2 Outflow	3453.50	**2.89**	0.40	19.25	**0.09**
W2.3	BC# 2	3762.00	**2.89**	**0.31**	20.75	**0.09**
W2.4	BC# 2 Inflow	3797.50	**2.89**	**0.31**	22.85	**0.09**
W2.5	BC# 3	3883.00	**2.89**	**0.31**	24.70	**0.09**
W2.6	BC# 3 Inflow	4877.50	**2.89**	0.50	17.65	**0.09**
W2.7	Cliff pond # 28	543.00	**2.89**	**0.31**	32.55	**0.09**
W2.8	Cliff pond Outflow	527.00	**2.89**	**0.31**	29.80	**0.09**
W2.10	H2O BC 1 ASC	3100.00	**2.89**	**0.31**	51.20	**0.09**
W2.11	H2O BC 1 BSC	2861.50	**2.89**	**0.31**	49.90	**0.09**
W2.12	Inflow Cliffs	547.00	**2.89**	**0.31**	37.75	**0.09**
w2.2 F	BC 1 Outflow - filtered	2946.50	**2.89**	**0.31**	46.70	**0.09**
W2.1 F	BC 2 Outflow - filtered	2606.00	**2.89**	**0.31**	11.70	**0.09**
W2.3 F	BC# 2 - filtered	3535.00	**2.89**	**0.31**	20.00	**0.09**
W2.4 F	BC# 2 Inflow - filtered	3594.00	**2.89**	**0.31**	18.25	**0.09**
W2.5 F	BC# 3 - filtered	3639.50	**2.89**	**0.31**	22.95	**0.09**
W2.6 F	BC# 3 Inflow - filtered	4473.50	**2.89**	**0.31**	13.05	**0.09**
W2.7 F	Cliff pond # 28 - filtered	536.00	**2.89**	0.40	33.20	**0.09**
W2.9 F	Cliff pond Outflow - filtered	522.00	**2.89**	**0.31**	31.05	**0.09**
W2.00 F	Dupe 1-Cliff pond # 28	532.50	**2.89**	**0.31**	32.20	**0.09**
W2.11 F	H2O BC 1 ASC - filtered	2845.50	**2.89**	**0.31**	47.00	**0.09**
W2.10 F	H2O BC 1 BSC - filtered	2792.00	**2.89**	**0.31**	48.85	**0.09**
W2.12 F	Inflow Cliffs - filtered	507.00	**2.89**	**0.31**	33.75	**0.09**
W2.? F	Nist water	322.00	79.50	6.80	547.50	16.55
Wcert	Certified Value	315.20	118.50	6.41	531.00	19.15
	MDL	0.43	2.89	0.31	0.09	0.09
	Average	**2381.01**	**10.58**	**0.83**	**71.66**	**1.51**
	s.d	**1506**	**27**	**2**	**138**	**5**

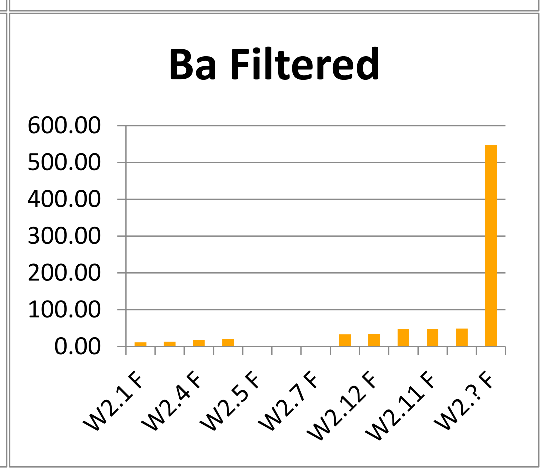




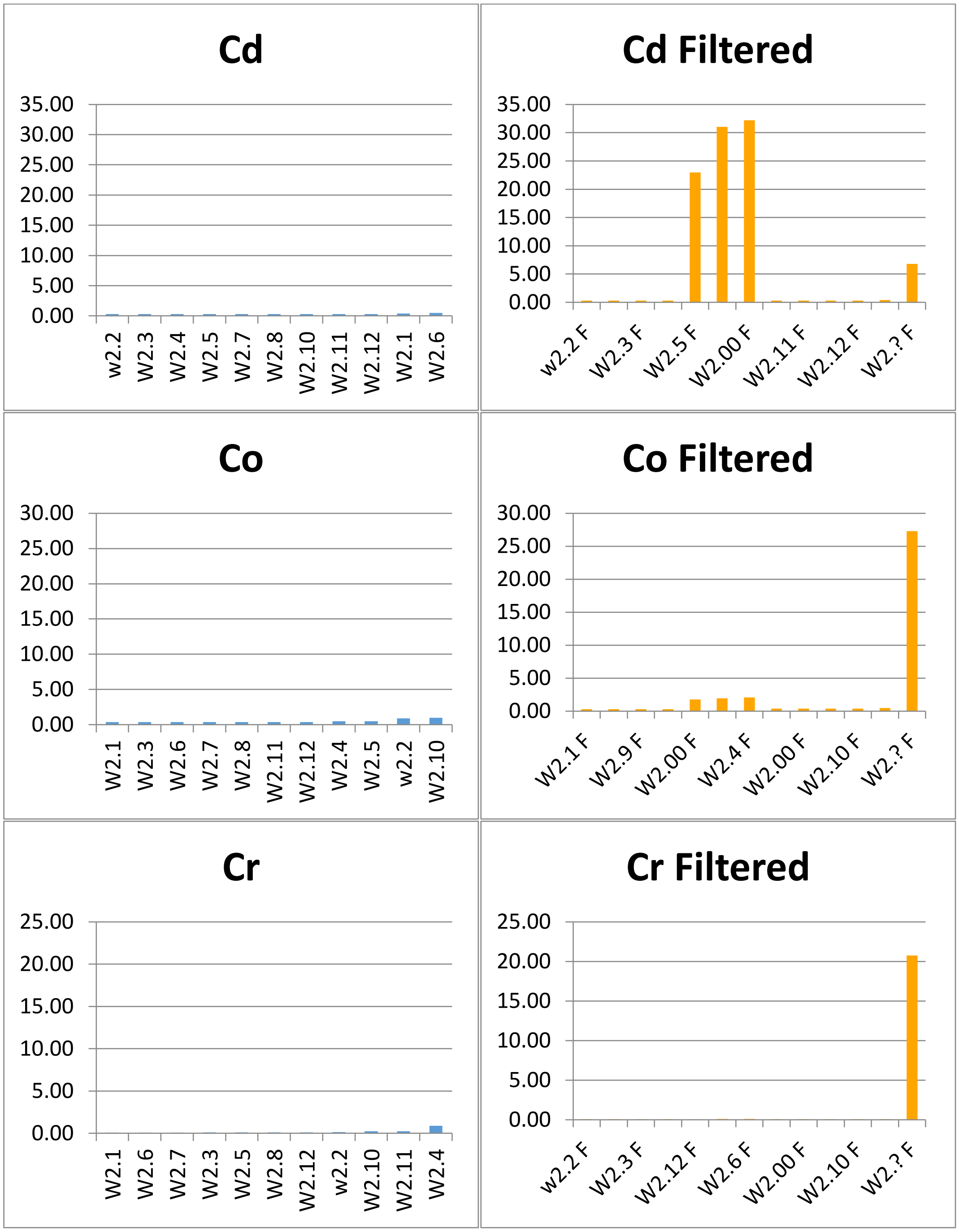


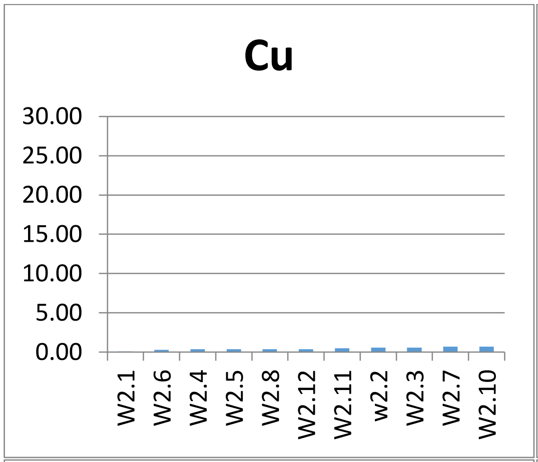


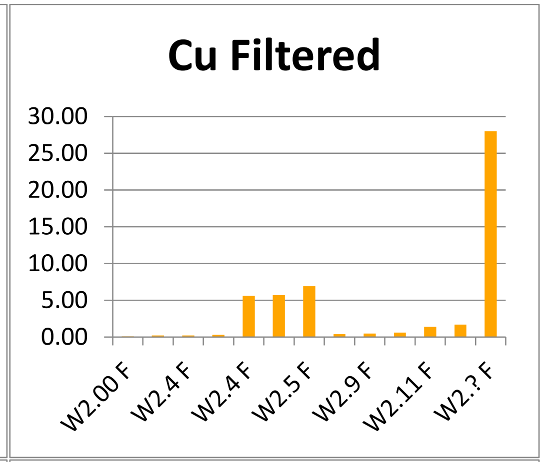


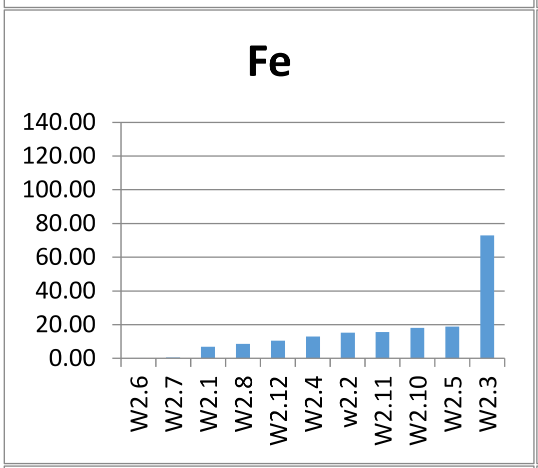


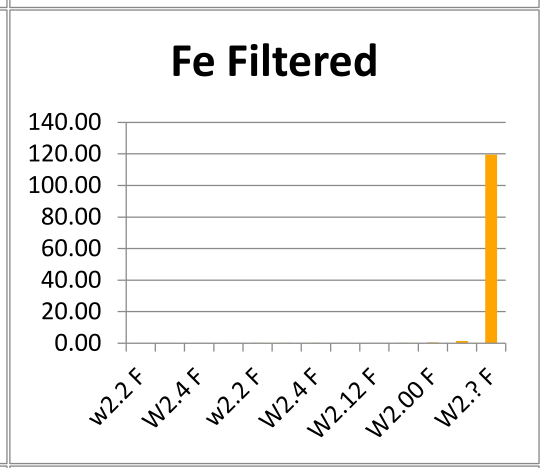
All Charts are in ug/L

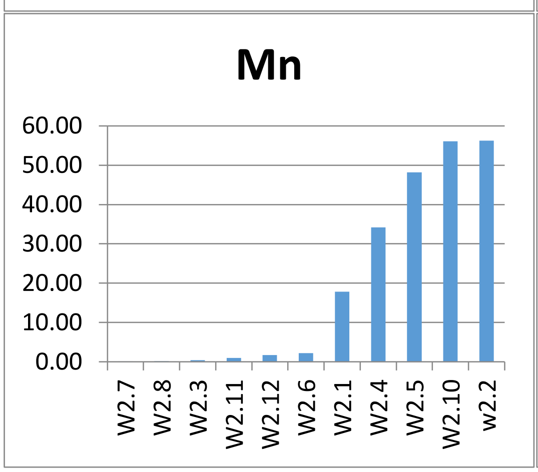

Cd
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
w2.2
W2.3
W2.4
W2.5
W2.7
W2.8
W2.10
W2.11
W2.12
W2.1
W2.6
Cd Filtered
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
w2.2 F
W2.3 F
W2.5 F
W2.00 F
W2.11 F
W2.12 F
W2.? F
Co
30.00
25.00
20.00
15.00
10.00
5.00
0.00
W2.1
W2.3
W2.6
W2.7
W2.8
W2.11
W2.12
W2.4
W2.5
w2.2
W2.10
Co Filtered
30.00
25.00
20.00
15.00
10.00
5.00
0.00
W2.1 F
W2.9 F
W2.00 F
W2.4 F
W2.00 F
W2.10 F
W2.? F
Cr
25.00
20.00
15.00
10.00
5.00
0.00
W2.1
W2.6
W2.7
W2.3
W2.5
W2.8
W2.12
w2.2
W2.10
W2.11
W2.4
Cr Filtered
25.00
20.00
15.00
10.00
5.00
0.00
w2.2 F
W2.3 F
W2.12 F
W2.6 F
W2.00 F
W2.10 F
W2.? F

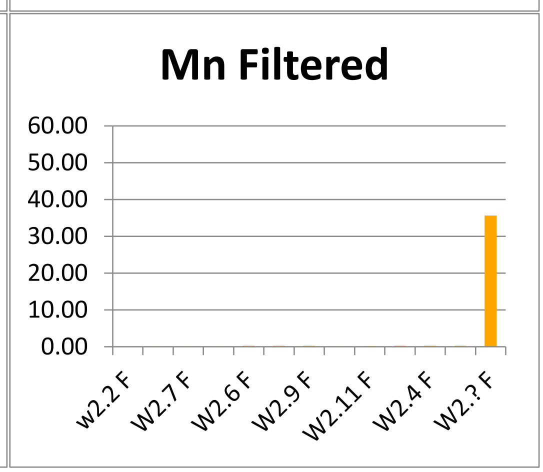

Cu
30.00
25.00
20.00
15.00
10.00
5.00
0.00
W2.1
W2.6
W2.4
W2.5
W2.8
W2.12
W2.11
w2.2
W2.3
W2.7
W2.10

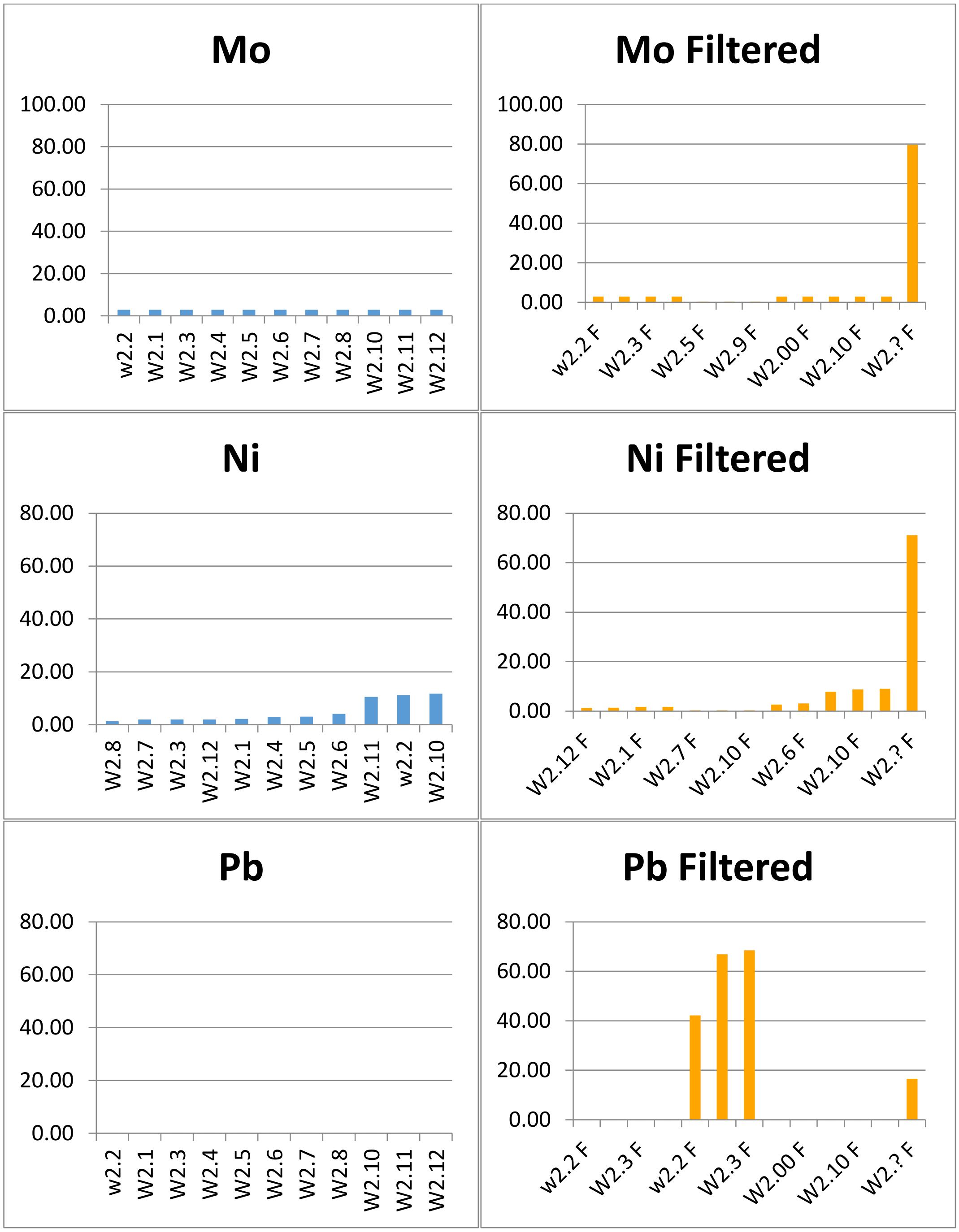

Cu Filtered
30.00
25.00
20.00
15.00
10.00
5.00
0.00
W2.00 F
W2.4 F
W2.4 F
W2.5 F
W2.9 F
W2.11 F
W2.? F

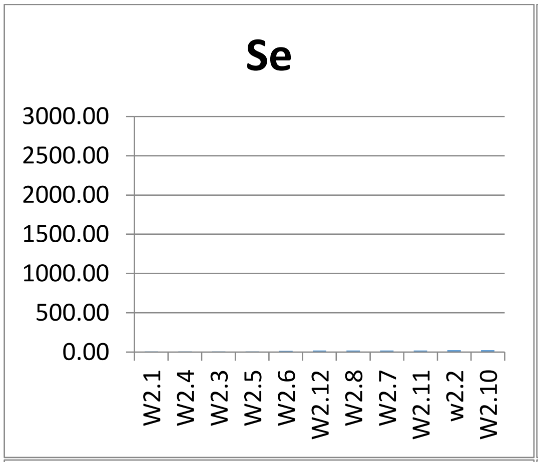

Fe
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
W2.6
W2.7
W2.1
W2.8
W2.12
W2.4
w2.2
W2.11
W2.10
W2.5
W2.3

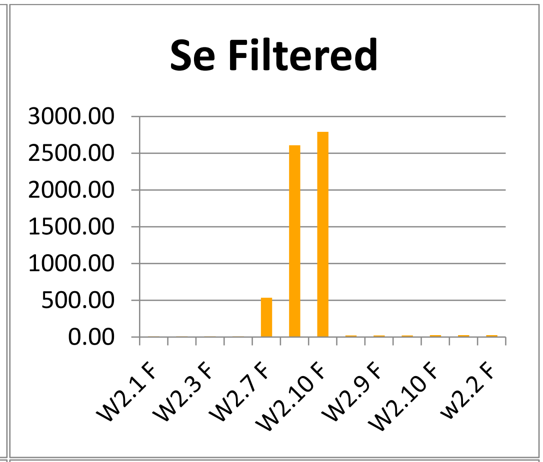

Fe Filtered
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
w2.2 F
W2.4 F
w2.2 F
W2.4 F
W2.12 F
W2.00 F
W2.? F

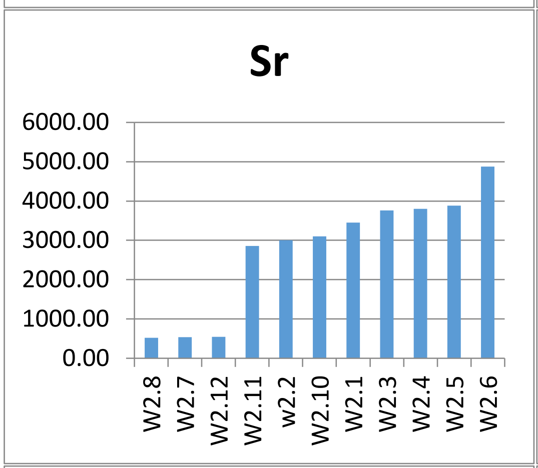

Mn
60.00
50.00
40.00
30.00
20.00
10.00
0.00
W2.7
W2.8
W2.3
W2.11
W2.12
W2.6
W2.1
W2.4
W2.5
W2.10
w2.2

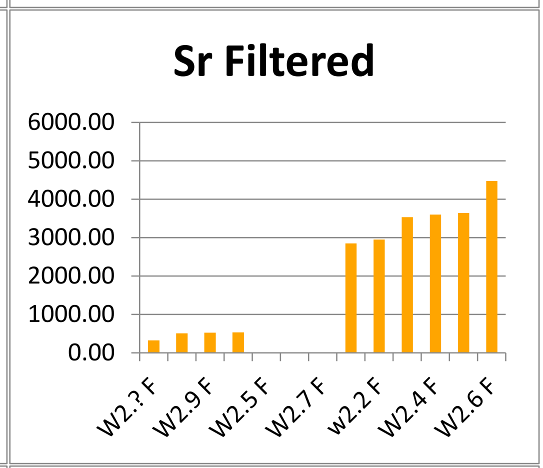

Mn Filtered
60.00
50.00
40.00
30.00
20.00
10.00
0.00
w2.2 F
W2.7 F
W2.6 F
W2.9 F
W2.11 F
W2.4 F
W2.? F

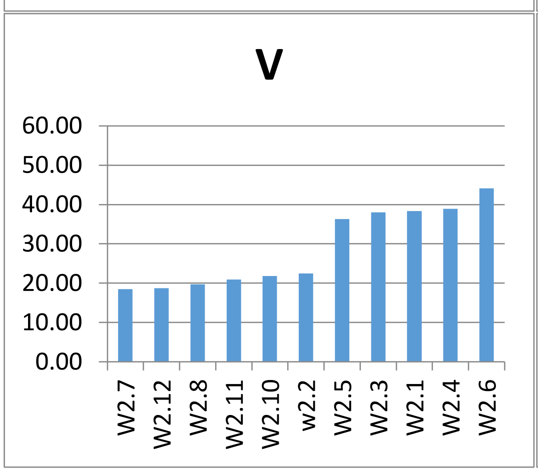

Mo
100.00
80.00
60.00
40.00
20.00
0.00
w2.2
W2.1
W2.3
W2.4
W2.5
W2.6
W2.7
W2.8
W2.10
W2.11
W2.12
Mo Filtered
100.00
80.00
60.00
40.00
20.00
0.00
w2.2 F
W2.3 F
W2.5 F
W2.9 F
W2.00 F
W2.10 F
W2.? F
Ni
80.00
60.00
40.00
20.00
0.00
W2.8
W2.7
W2.3
W2.12
W2.1
W2.4
W2.5
W2.6
W2.11
w2.2
W2.10
Ni Filtered
80.00
60.00
40.00
20.00
0.00
W2.12 F
W2.1 F
W2.7 F
W2.10 F
W2.6 F
W2.10 F
W2.? F
Pb
80.00
60.00
40.00
20.00
0.00
w2.2
W2.1
W2.3
W2.4
W2.5
W2.6
W2.7
W2.8
W2.10
W2.11
W2.12
Pb Filtered
80.00
60.00
40.00
20.00
0.00
w2.2 F
W2.3 F
w2.2 F
W2.3 F
W2.00 F
W2.10 F
W2.? F

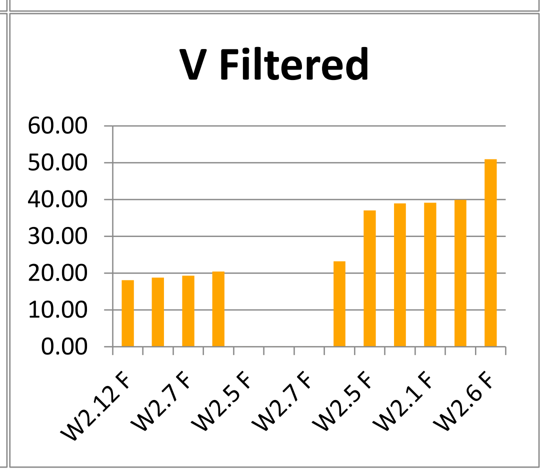

Se
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
W2.1
W2.4
W2.3
W2.5
W2.6
W2.12
W2.8
W2.7
W2.11
w2.2
W2.10

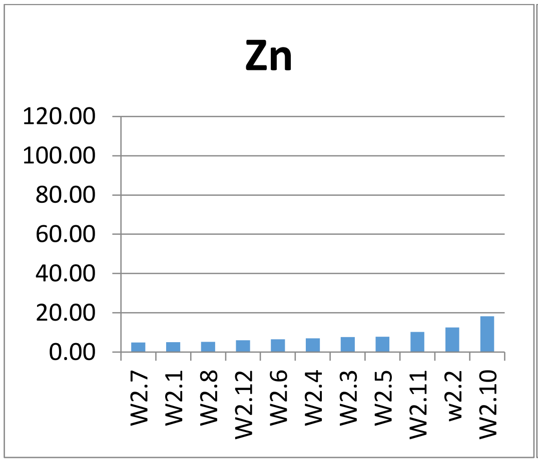

Se Filtered
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
W2.1 F
W2.3 F
W2.7 F
W2.10 F
W2.9 F
W2.10 F
w2.2 F

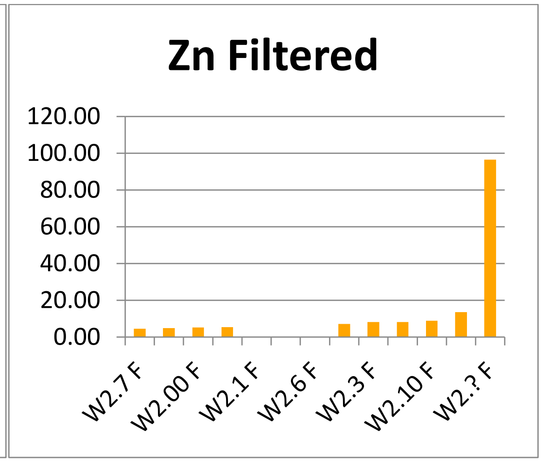

Sr
6000.00
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
W2.8
W2.7
W2.12
W2.11
w2.2
W2.10
W2.1
W2.3
W2.4
W2.5
W2.6


Sr Filtered
6000.00
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
W2.9 F
W2.5 F
W2.7 F
w2.2 F
W2.4 F
W2.6 F


V
60.00
50.00
40.00
30.00
20.00
10.00
0.00
W2.7
W2.12
W2.8
W2.11
W2.10
w2.2
W2.5
W2.3
W2.1
W2.4
W2.6


V Filtered
60.00
50.00
40.00
30.00
20.00
10.00
0.00
W2.12 F
W2.7 F
W2.5 F
W2.7 F
W2.5 F
W2.1 F
W2.6 F





4. Sediment cores profiles all elements Cliff's and BC 1

	Sample ID	cm from surface	Core	B	V	Cr	Mn	Co
MK-001	Cliff Pond Core - 2-4	8-10	Cliff pond	**0.27**	10.59	10.39	491.4	10.04
MK-002	Cliff Pond Core - 4-6	6-8	Cliff pond	**0.27**	15.33	13.37	292.6	8.29
MK-003	Cliff Pond Core - 6-8	4-6	Cliff pond	**0.27**	15.74	14.41	229.7	11.50
MK-004	Cliff Pond Core - 8-10	2-4	Cliff pond	**0.27**	13.69	12.76	301.1	10.13
MK-005	Cliff Pond Core - 10-12	0-2	Cliff pond	**0.27**	13.57	11.71	769.1	8.77
MK-006	BC #1- 0-2	22-24	BC#1	**0.27**	13.77	11.95	289.5	10.41
MK-007	BC #1- 2-4	20-22	BC#1	**0.27**	17.48	16.44	283.4	10.30
MK-008	BC #1- 4-6	18-20	BC#1	**0.27**	15.56	14.56	326.6	10.48
MK-009	BC #1- 6-8	16-18	BC#1	**0.27**	16.05	16.37	336.0	12.25
MK-010	BC #1- 8-10	14-16	BC#1	**0.27**	12.06	13.30	356.0	13.40
MK-011	BC #1- 10-12	12-14	BC#1	1.71	15.29	13.84	340.6	13.35
MK-012	BC #1- 12-14	10-12	BC#1	0.60	13.46	12.69	350.7	13.60
MK-013	BC #1- 14-16	8-10	BC#1	1.68	14.76	13.20	299.2	11.64
MK-014	BC #1- 16-18	6-8	BC#1	0.58	13.59	11.64	344.7	12.88
MK-015	BC #1- 18-20	4-6	BC#1	1.71	14.88	13.13	343.6	12.40
MK-016	BC #1- 20-22	2-4	BC#1	0.68	13.62	12.73	365.3	13.60
MK-017	BC #1- 22-24	0-2	BC#1	**0.27**	11.55	11.33	374.1	13.96
MK-018	Dupe 1- Cliff Pond Core -6-8	6-8	Cliff pond	**0.27**	9.85	6.82	318.4	9.25
MK-019	Dupe 2- Cliff Pond Core -2-4	2-4	Cliff pond	**0.27**	11.98	10.26	337.7	11.55
MK-020	Dupe 3-BC #1- 2-4	20-22	BC#1	**0.27**	13.24	12.18	308.8	11.27
MK-021	Dupe 4-BC #1- 18-20	4-6	BC#1	1.28	13.50	12.51	354.5	12.87
MK-022	SRM1- Montana II			4.05	23.37	12.04	326.8	4.89
MK-023	SRM2- Montana II			3.52	21.81	12.56	326.8	4.87
	Average			**0.85**	**14.55**	**12.62**	**350.72**	**10.94**
	Sd			**1**	**3**	**2**	**100**	**2**
	Certified acid leachable				28	15	460	7.5
	Method detection limit			**0.27**	**0.02**	**0.07**	**0.03**	**0.03**

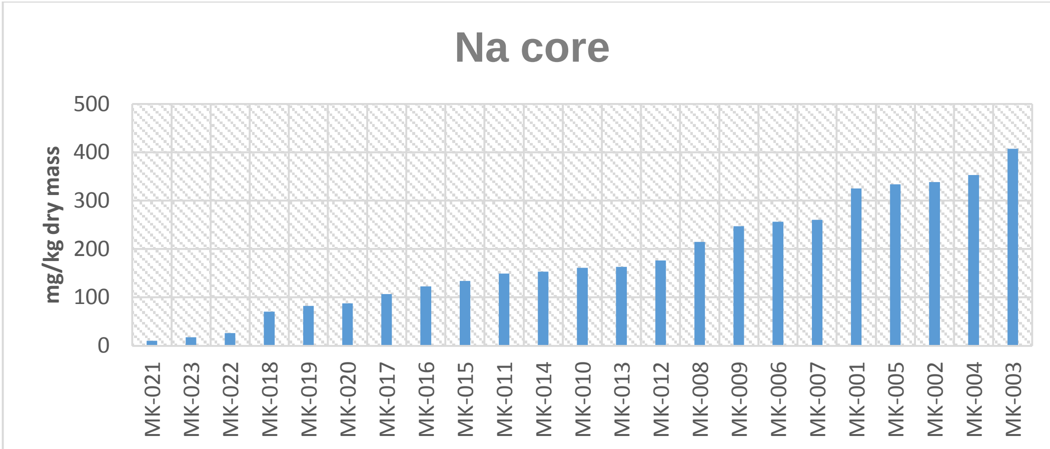
MK-001	Cliff Pond Core - 2-4	8-10	Cliff pond	**Ni**	**Cu**	**Zn**	**As**	**Se**
MK-002	Cliff Pond Core - 4-6	6-8	Cliff pond	25.29	20.29	67.49	3.62	0.75
MK-003	Cliff Pond Core - 6-8	4-6	Cliff pond	27.43	23.67	68.44	4.24	0.60
MK-004	Cliff Pond Core - 8-10	2-4	Cliff pond	36.68	25.50	77.68	4.06	1.37
MK-005	Cliff Pond Core - 10-12	0-2	Cliff pond	35.68	24.20	85.93	4.00	7.44
MK-006	BC #1- 0-2	22-24	BC#1	39.17	19.68	109.58	3.28	17.01
MK-007	BC #1- 2-4	20-22	BC#1	28.81	22.76	78.10	4.42	0.66
MK-008	BC #1- 4-6	18-20	BC#1	35.66	24.60	91.23	4.84	1.26
MK-009	BC #1- 6-8	16-18	BC#1	34.76	25.52	94.35	4.84	1.49
MK-010	BC #1- 8-10	14-16	BC#1	38.26	26.14	94.28	5.16	0.96
MK-011	BC #1- 10-12	12-14	BC#1	36.31	26.56	93.61	5.45	0.74
MK-012	BC #1- 12-14	10-12	BC#1	40.45	28.42	99.74	5.74	0.92
MK-013	BC #1- 14-16	8-10	BC#1	38.62	27.09	95.86	5.42	0.90
MK-014	BC #1- 16-18	6-8	BC#1	34.21	24.03	87.26	5.11	0.96
MK-015	BC #1- 18-20	4-6	BC#1	36.36	26.88	96.54	5.80	1.15
MK-016	BC #1- 20-22	2-4	BC#1	34.51	24.82	88.82	5.78	0.90
MK-017	BC #1- 22-24	0-2	BC#1	36.96	26.55	93.74	5.78	0.82
MK-018	Dupe 1- Cliff Pond Core -6-8	6-8	Cliff pond	35.93	25.74	91.78	5.50	0.72
MK-019	Dupe 2- Cliff Pond Core -2-4	2-4	Cliff pond	25.31	23.35	67.39	3.76	0.53
MK-020	Dupe 3-BC #1- 2-4	20-22	BC#1	38.71	26.43	95.80	4.36	7.44
MK-021	Dupe 4-BC #1- 18-20	4-6	BC#1	35.75	25.74	94.36	4.77	1.39
MK-022	SRM1- Montana II			34.75	25.05	90.56	5.63	0.84
MK-023	SRM2- Montana II			18.24	133.77	400.04	103.65	1.80
	Average			17.59	134.75	394.83	102.52	1.81
	Sd			**33.28**	**34.41**	**115.54**	**13.38**	**2.28**
				6	**31**	**88**	**28**	**4**
	Certified acid leachable							
	Method detection limit			15	130	350	89	1.7
				0.12	**1.07**	**2.83**	**0.03**	**0.03**

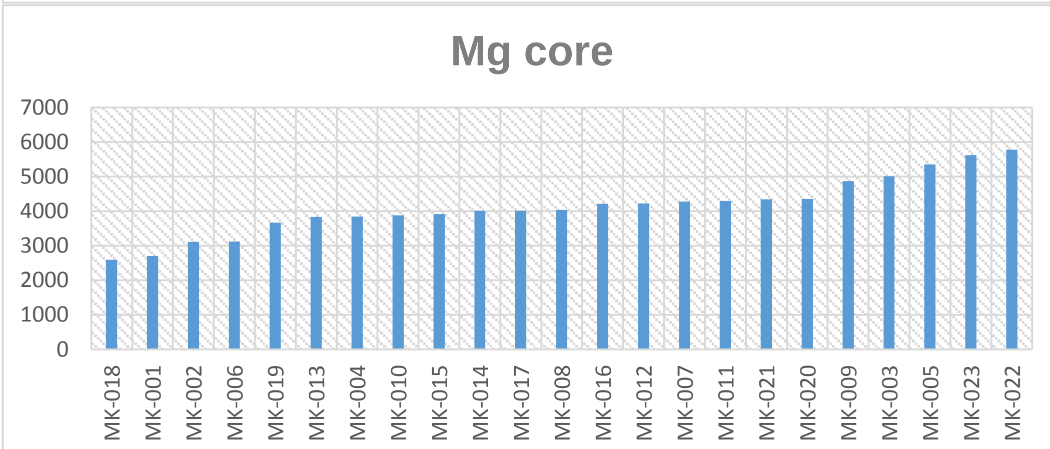
				Sr	Mo	Cd	Ba	Pb
MK-001	Cliff Pond Core - 2-4	8-10	Cliff pond	6.48	**0.72**	**0.02**	70.54	14.39
MK-002	Cliff Pond Core - 4-6	6-8	Cliff pond	9.75	**0.72**	**0.02**	114.92	16.93
MK-003	Cliff Pond Core - 6-8	4-6	Cliff pond	10.58	**0.72**	0.04	149.91	19.39
MK-004	Cliff Pond Core - 8-10	2-4	Cliff pond	18.59	**0.72**	0.02	116.10	17.65
MK-005	Cliff Pond Core - 10-12	0-2	Cliff pond	94.92	**0.72**	0.14	112.17	14.07
MK-006	BC #1- 0-2	22-24	BC#1	12.93	**0.72**	**0.02**	121.82	16.56
MK-007	BC #1- 2-4	20-22	BC#1	13.41	**0.72**	**0.02**	116.17	18.64
MK-008	BC #1- 4-6	18-20	BC#1	18.85	**0.72**	**0.02**	111.87	18.12
MK-009	BC #1- 6-8	16-18	BC#1	16.51	**0.72**	**0.02**	114.55	19.59
MK-010	BC #1- 8-10	14-16	BC#1	11.55	**0.72**	**0.02**	100.94	19.07
MK-011	BC #1- 10-12	12-14	BC#1	13.13	**0.72**	0.10	136.20	21.62
MK-012	BC #1- 12-14	10-12	BC#1	12.65	0.74	0.08	115.16	20.24
MK-013	BC #1- 14-16	8-10	BC#1	12.54	**0.72**	0.08	106.93	17.87
MK-014	BC #1- 16-18	6-8	BC#1	11.68	0.89	0.10	101.07	19.33
MK-015	BC #1- 18-20	4-6	BC#1	12.05	0.73	0.10	111.32	18.42
MK-016	BC #1- 20-22	2-4	BC#1	12.43	0.84	0.10	116.56	19.79
MK-017	BC #1- 22-24	0-2	BC#1	12.32	0.74	0.10	110.95	21.87
MK-018	Dupe 1- Cliff Pond Core -6-8	6-8	Cliff pond	5.62	**0.72**	0.08	86.99	16.02
MK-019	Dupe 2- Cliff Pond Core -2-4	2-4	Cliff pond	18.44	0.81	0.14	118.42	19.80
MK-020	Dupe 3-BC #1- 2-4	20-22	BC#1	12.28	0.83	0.12	97.48	18.66

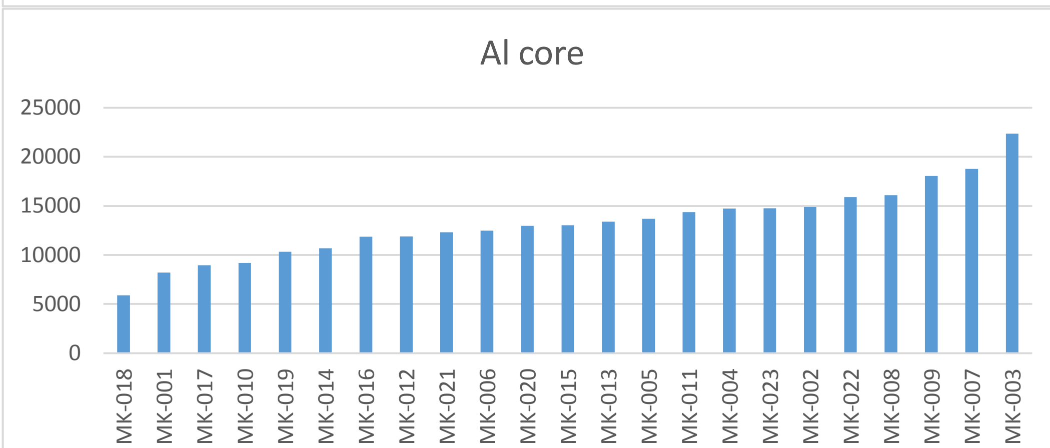
				Sr	Mo	Cd	Ba	Pb
MK-021	Dupe 4-BC #1- 18-20	4-6	BC#1	11.63	1.08	0.08	102.77	18.09
MK-022	SRM1- Montana II			13.42	1.11	44.64	181.39	1048.35
MK-023	SRM2- Montana II			13.26	1.09	44.77	178.91	1038.77
	Average			**16.30**	**0.79**	**3.95**	**117.09**	**107.53**
	Sd			**17.05**	**0.13**	**12.58**	**24.54**	**288.87**
	Certified acid leachable					47	190	1300
	Method detection limit			**0.06**	**0.72**	**0.02**	**0.03**	**0.16**

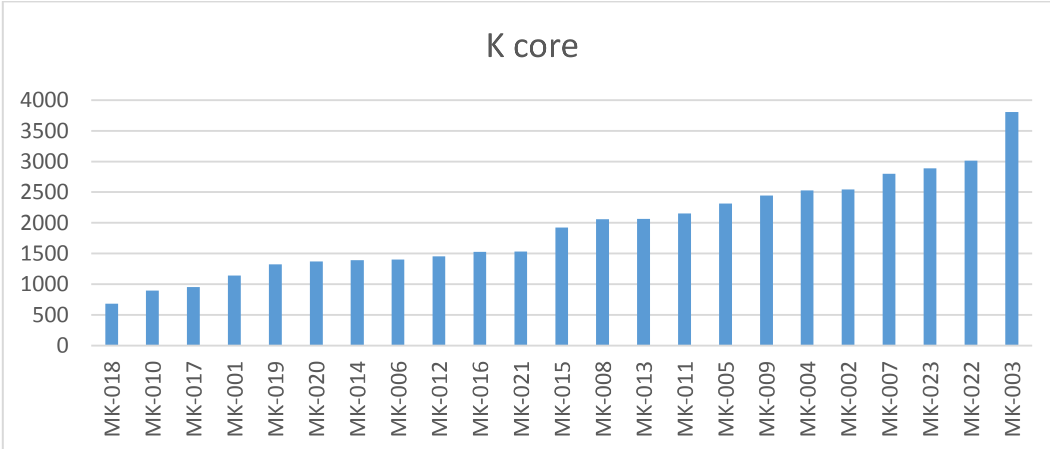
5. Sediment profiles all elements continued

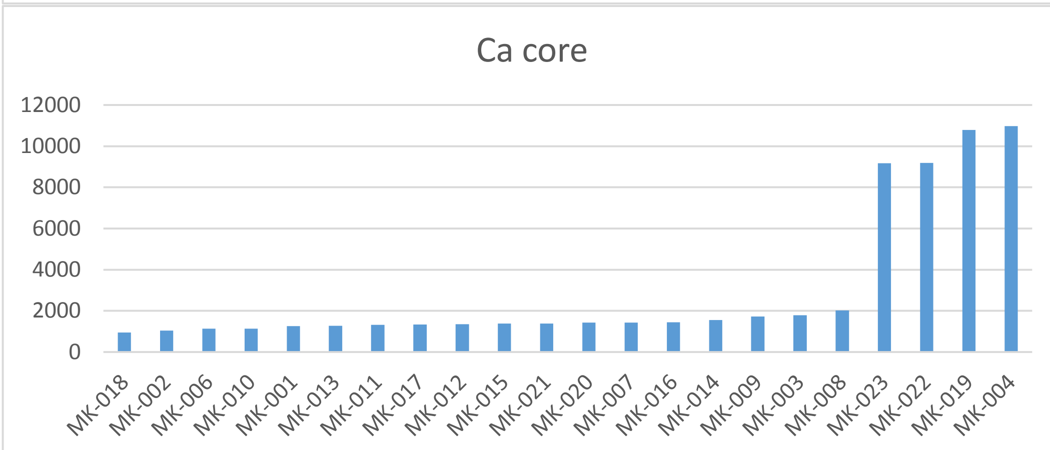
		mg/kg dry mass					
		Na	Mg	Al	K	Ca	Fe
MK-001	Cliff Pond Core - 2-4	325.53	2696.32	8189.33	1141.31	1262.55	21649.28
MK-002	Cliff Pond Core - 4-6	338.50	3108.59	14898.57	2545.74	1039.98	22712.81
MK-003	Cliff Pond Core - 6-8	407.05	5006.97	22355.05	3805.54	1788.20	32895.00
MK-004	Cliff Pond Core - 8-10	353.03	3840.80	14742.29	2529.35	10973.13	23820.90
MK-005	Cliff Pond Core - 10-12	333.66	5347.69	13668.51	2317.27	66357.17	17856.58
MK-006	BC #1- 0-2	256.74	3120.88	12485.51	1400.60	1135.26	21958.04
MK-007	BC #1- 2-4	260.51	4273.47	18772.17	2800.48	1431.13	25313.93
MK-008	BC #1- 4-6	214.88	4031.75	16113.10	2059.52	2021.83	24861.11
MK-009	BC #1- 6-8	246.95	4872.92	18044.83	2445.47	1729.64	26956.17
MK-010	BC #1- 8-10	161.26	3874.18	9171.81	894.68	1139.16	20864.03
MK-011	BC #1- 10-12	148.98	4296.38	14378.93	2154.09	1325.67	26729.56
MK-012	BC #1- 12-14	176.06	4216.29	11884.09	1453.10	1358.30	25512.85
MK-013	BC #1- 14-16	162.97	3826.64	13399.24	2063.11	1283.60	22848.01
MK-014	BC #1- 16-18	152.99	4007.55	10691.44	1388.44	1562.29	26008.34
MK-015	BC #1- 18-20	133.84	3917.06	13044.42	1924.72	1379.52	23211.48
MK-016	BC #1- 20-22	122.88	4211.05	11853.18	1525.63	1448.04	25912.63
MK-017	BC #1- 22-24	107.08	4007.98	8941.18	953.34	1341.77	23449.65
MK-018	Dupe 1- Cliff Pond Core -6-8	70.40	2590.47	5894.80	682.82	954.91	20565.55
MK-019	Dupe 2- Cliff Pond Core -2-4	82.71	3661.67	10323.73	1323.13	10791.55	23529.41
MK-020	Dupe 3-BC #1- 2-4	87.44	4348.17	12955.09	1370.63	1423.89	24582.67
MK-021	Dupe 4-BC #1- 18-20	**10.00**	4344.70	12317.97	1530.42	1379.61	23259.53
MK-022	SRM1- Montana II	26.45	5780.54	15895.01	3015.59	9184.92	17448.19
MK-023	SRM2- Montana II	17.50	5618.29	14767.40	2888.67	9165.01	16763.42
	Average	**182.50**	**4130.45**	**13251.64**	**1922.33**	**5716.40**	**23422.14**
	s.d	**115.07**	**823.84**	**3666.27**	**786.79**	**13639.59**	**3499.93**
	Acid Leachable	140-210	5000-6600	9800-15000	3300-4600	14000-17001	14000-18002
	MDL	**10**	**7.89**	**26.32**	**221.14**	**20.58**	**37.82**

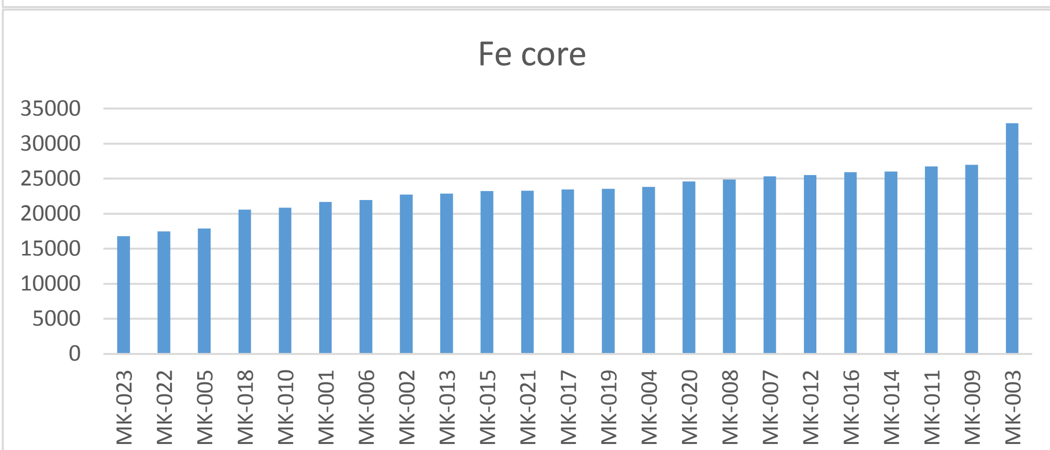

Na core
mg/kg dry mass
500
400
300
200
100
0
MK-021
MK-023
MK-022
MK-018
MK-019
MK-020
MK-017
MK-016
MK-015
MK-011
MK-014
MK-010
MK-013
MK-012
MK-008
MK-009
MK-006
MK-007
MK-001
MK-005
MK-002
MK-004
MK-003


Mg core
7000
6000
5000
4000
3000
2000
1000
0
MK-018
MK-001
MK-002
MK-006
MK-019
MK-013
MK-004
MK-010
MK-015
MK-014
MK-017
MK-008
MK-016
MK-012
MK-007
MK-011
MK-021
MK-020
MK-009
MK-003
MK-005
MK-023
MK-022


Al core
25000
20000
15000
10000
5000
0
MK-018
MK-001
MK-017
MK-010
MK-019
MK-014
MK-016
MK-012
MK-021
MK-006
MK-020
MK-015
MK-013
MK-005
MK-011
MK-004
MK-023
MK-002
MK-022
MK-008
MK-009
MK-007
MK-003


K core
4000
3500
3000
2500
2000
1500
1000
500
0
MK-018
MK-010
MK-017
MK-001
MK-019
MK-020
MK-014
MK-006
MK-012
MK-016
MK-021
MK-015
MK-008
MK-013
MK-011
MK-005
MK-009
MK-004
MK-002
MK-007
MK-023
MK-022
MK-003


Ca core
12000
10000
8000
6000
4000
2000
0
MK-018
MK-002
MK-006
MK-010
MK-001
MK-013
MK-011
MK-017
MK-012
MK-015
MK-021
MK-020
MK-007
MK-016
MK-014
MK-009
MK-003
MK-008
MK-023
MK-022
MK-019
MK-004


Fe core
35000
30000
25000
20000
15000
10000
5000
0
MK-023
MK-022
MK-005
MK-018
MK-010
MK-001
MK-006
MK-002
MK-013
MK-015
MK-021
MK-017
MK-019
MK-004
MK-020
MK-008
MK-007
MK-012
MK-016
MK-014
MK-011
MK-009
MK-003

6. Vegetation/ its parts, algae and debris all [elements]

		mg/kg dry mass				
	Sample	**B**	**Na**	**Mg**	**Al**	**K**
P1	BC #1	3.85	85	4972	13736	2771
P2	BC #1 Pond weed	11.00	994	3779	1279	24985
P3	BC #1 Pond weed Wash	BDL	352	8107	19596	6197
P4	BC #1 Before S. Curtain	4.02	97	5399	13907	2760
P5	BC #1 Leaf Debris	49.58	145	4721	2138	2328
P6	BC #1 Leaf Debris Wash	BDL	273	9362	22440	4301
P7	BC #1 BSC Floating cattail Debris	13.69	202	5105	6457	2092
P8	BC #1 BSC Floating cattail Debris Wash	6.11	191	8855	45671	2725
P9	BC# 2 Plant 1	14.61	343	7893	861	2963
P10	BC# 2 Plant 1 Wash	3.24	297	2759	2522	1365
P11	BC# 2 Chara	42.04	429	2680	291	6178
P12	BC# 2 Chara Wash	13.29	260	1488	3586	368
P13	BC #2 Potpus	48.08	302	1427	861	1984
P14	BC #2 Potpus Wash	5.30	340	3492	1545	1336
P15	BC #2 nearshor crustose algae	9.56	228	2713	824	471
P16	BC #2 nearshor crustose algae Wash	4.59	208	1698	2825	326
P17	BC #2 potpus	31.06	273	1998	1023	1234
P18	BC #2 potpus Wash	10.37	230	1668	1156	665
P19	BC #2 dead cattail stock	4.07	221	2880	520	387
P20	BC #2 dead cattail stock Wash	BDL	182	2166	4612	250
P21	BC #2 dead cattail surface	BDL	153	892	614	256
P22	BC #2 dead cattail surface Wash	BDL	186	969	6064	242
P23	BC #1 cattail- Root	5.52	3920	1959	842	59794
P24	BC #1 cattail- Rhizo	5.43	1258	2044	728	24305
P25	BC #1 cattail- leaf	8.96	733	2375	418	36188
P26	BC #2 cattail- Root	11.98	1085	4490	1412	5993
P27	BC #2 cattail- Rhizo	4.79	450	3058	107	17369
P28	BC #2 cattail- leaf	7.24	48	3909	103	14156
P29	Dupe 1-BC #1	BDL	69	4318	11009	2380
P30	Dupe 2- BC# 2 Chara	28.93	316	1981	173	4817
P31	Dupe 3- BC #2 potpus	38.52	236	1084	489	1458
P32	Dupe 4- BC #2 cattail- leaf	9.06	55	4414	107	15336
P33	Dupe 5- BC #2 cattail- Root	15.51	1153	5294	3028	6332
P34	BC #3 Algae	6.28	395	1896	696	560
P35	BC #3 Algae Wash	BDL	666	9254	2853	1658
P36	BC #3 Cara Inflow	11.04	309	3615	3035	4874
P37	BC #3 Cara Inflow Wash	7.71	434	3755	3069	1828
P38	BC #3 Aquatic moss	95.49	298	2053	1544	2177
P39	BC #3 Aquatic moss Wash	7.26	350	3900	6793	540
P40	BC #3 Cara	14.16	184	3188	3388	2769
P41	BC #3 Cara Wash	16.29	211	3650	4363	2651
P42	BC #3 Stupee	189.30	881	3784	2593	13950
P43	BC #3 Stupee Wash	195.92	497	6137	5932	4545
P44	Cliff pond Potpus	42.33	1578	1900	286	10054
P45	Cliff pond Potpus Wash	BDL	180	522	281	383
P46	Cliff pond grassy plant	12.08	546	4679	1121	7802
P47	Cliff pond grassy plant Wash	BDL	313	5194	3561	2307
P48	Cliff pond weed #2	7.82	299	4815	954	3892
P49	Cliff pond weed #2 Wash	BDL	342	5309	2122	2823
P50	Cliff pond potnat	8.91	433	4937	854	4858
P51	Cliff pond potnat Wash	BDL	278	5264	1869	2294

	mg/kg dry mass					
	Sample	**B**	**Na**	**Mg**	**Al**	**K**
P52	Cliff pond algae bottom	7.52	177	5038	1735	652
P53	Cliff pond algae bottom Wash	BDL	152	4681	2801	1015
P54	Cliff pond floating algae	3.74	134	4191	5629	1471
P55	Cliff pond floating algae Wash	BDL	128	5503	22254	2538
P56	Cliff pond tire algae	34.86	190	3409	241	2808
P57	Cliff pond tire algae Wash	BDL	355	9582	7695	891
P58	Cliff pond- Root	BDL	2320	3715	395	27696
P59	Cliff pond- leaf	5.78	319	1395	17	14902
P60	Cliff pond plant - Root	8.91	6053	2927	854	15747
P61	Cliff pond plant - leaf	7.03	1370	6194	49	20846
P62	BC #1 Scripus- Root	5.73	1612	6526	1522	25587
P63	BC #1 Scripus- leaf	8.08	218	8469	430	23424
P64	Dupe 1-BC #3 Cara Inflow	9.57	265	3177	1718	4024
P65	Dupe 2- BC #3 Stupee	219.43	989	4231	1348	13976
P66	Dupe 3- Cliff pond potnat	8.82	505	5800	1040	5472
P67	Dupe 4- Cliff pond algae bottom	7.45	195	5858	2130	794
P68	Dupe 5- Cliff pond- leaf	4.19	393	1717	30	18702
P69	SRM 1 - TORT 2	3.89	12025	1081	9	8359
P70	SRM 2 -TORT 2	4.32	12541	1122	11	8480
P71	SRM 1 - TORT 2	11.56	13336	1196	15	8736
P72	SRM 2 -TORT 2	12.38	13901	1254	17	8943
	Average	**24.36**	**1259.47**	**3900.90**	**3697.15**	**7490.84**
	s.d	**45**	**2992**	**2236**	**6950**	**10153**
	Certified Value					
	MDL	2.75	2.48	4.11	3.55	5.10

Method- Microwave acid digestion ICP-MS

	mg/kg dry mass					
	Sample	**Ca**	**V**	**Cr**	**Mn**	**Fe**
P1	BC #1	1323	21	19	535	26133
P2	BC #1 Pond weed	6205	2	2	157	1564
P3	BC #1 Pond weed Wash	37143	19	16	569	17592
P4	BC #1 Before S. Curtain	1521	23	20	579	26535
P5	BC #1 Leaf Debris	11459	3	2	272	2244
P6	BC #1 Leaf Debris Wash	9121	17	13	499	13793
P7	BC #1 BSC Floating cattail Debris	33153	10	13	2111	6551
P8	BC #1 BSC Floating cattail Debris Wash	40545	18	13	1449	14826
P9	BC# 2 Plant 1	98468	1	1	1360	948
P10	BC# 2 Plant 1 Wash	130942	2	1	1625	1357
P11	BC# 2 Chara	138849	0	3	1189	541
P12	BC# 2 Chara Wash	141624	1	1	1846	1797
P13	BC #2 Potpus	161178	1	10	811	977
P14	BC #2 Potpus Wash	136934	2	1	1795	1469
P15	BC #2 nearshor crustose algae	163875	1	5	608	682
P16	BC #2 nearshor crustose algae Wash	159944	1	1	659	888
P17	BC #2 potpus	148153	1	4	1103	1162
P18	BC #2 potpus Wash	130814	1	1	1201	1414
P19	BC #2 dead cattail stock	139916	1	2	447	465
P20	BC #2 dead cattail stock Wash	153521	1	1	483	653
P21	BC #2 dead cattail surface	111234	1	2	436	1172
P22	BC #2 dead cattail surface Wash	139128	1	1	447	932
P23	BC #1 cattail- Root	2181	2	2	1288	4825
P24	BC #1 cattail- Rhizo	1577	1	2	175	3219

		mg/kg dry mass				
	Sample	**Ca**	**V**	**Cr**	**Mn**	**Fe**
P25	BC #1 cattail- leaf	2252	1	1	203	732
P26	BC #2 cattail- Root	33207	3	3	4979	34587
P27	BC #2 cattail- Rhizo	3199	0	0	262	2284
P28	BC #2 cattail- leaf	5073	0	1	810	925
P29	Dupe 1-BC #1	1384	17	15	472	22580
P30	Dupe 2- BC# 2 Chara	105416	0	2	903	421
P31	Dupe 3- BC #2 potpus	112384	1	6	605	703
P32	Dupe 4- BC #2 cattail- leaf	4898	0	1	894	959
P33	Dupe 5- BC #2 cattail- Root	39768	6	6	5929	39489
P34	BC #3 Algae	126197	1	1	27	433
P35	BC #3 Algae Wash	69892	2	2	108	1609
P36	BC #3 Cara Inflow	97667	4	6	2059	2469
P37	BC #3 Cara Inflow Wash	119403	4	3	1443	2286
P38	BC #3 Aquatic moss	110178	2	16	131	778
P39	BC #3 Aquatic moss Wash	127605	2	1	143	775
P40	BC #3 Cara	60798	4	7	2583	2313
P41	BC #3 Cara Wash	61351	6	4	1739	3857
P42	BC #3 Stupee	28048	4	5	1502	2262
P43	BC #3 Stupee Wash	44760	5	5	2542	6312
P44	Cliff pond Potpus	89418	0	3	72	198
P45	Cliff pond Potpus Wash	72382	0	0	43	172
P46	Cliff pond grassy plant	107259	2	6	254	855
P47	Cliff pond grassy plant Wash	139636	BDL	BDL	253	991
P48	Cliff pond weed #2	111190	1	3	249	779
P49	Cliff pond weed #2 Wash	132855	1	1	333	892
P50	Cliff pond potnat	88161	1	5	128	817
P51	Cliff pond potnat Wash	105301	2	1	217	1464
P52	Cliff pond algae bottom	95446	2	2	313	2222
P53	Cliff pond algae bottom Wash	84398	3	BDL	510	2637
P54	Cliff pond floating algae	54538	8	6	1441	7897
P55	Cliff pond floating algae Wash	38845	10	7	1306	11339
P56	Cliff pond tire algae	93659	0	1	85	287
P57	Cliff pond tire algae Wash	85061	3	3	262	756
P58	Cliff pond- Root	2681	2	2	136	2768
P59	Cliff pond- leaf	816	0	0	170	61
P60	Cliff pond plant - Root	5871	2	3	466	5762
P61	Cliff pond plant - leaf	4974	0	0	99	114
P62	BC #1 Scripus- Root	2030	3	6	1044	1917
P63	BC #1 Scripus- leaf	5155	1	6	637	711
P64	Dupe 1-BC #3 Cara Inflow	91040	2	4	1861	2092
P65	Dupe 2- BC #3 Stupee	34086	2	4	1706	2263
P66	Dupe 3- Cliff pond potnat	132319	1	6	148	959
P67	Dupe 4- Cliff pond algae bottom	130738	3	2	359	2399
P68	Dupe 5- Cliff pond- leaf	1221	BDL	0	204	73
P69	SRM 1 - TORT 2	2426	2	1	12	92
P70	SRM 2 -TORT 2	2488	2	1	13	95
P71	SRM 1 - TORT 2	2006	2	1	14	107
P72	SRM 2 -TORT 2	2100	2	1	15	112
	Average	**69033**	**4**	**4**	**852**	**4283**
	s.d	**55958**	**5**	**5**	**1031**	**7972**
	Certified Value		1.64	0.77	13.60	105.00
	MDL	40.64	0.14	0.18	0.40	1.86

Method- Microwave acid digestion ICP-MS

		mg/kg dry mass				
	Sample	Co	Ni	Cu	Zn	As
P1	BC #1	20.26	34.89	25.69	90.93	5.26
P2	BC #1 Pond weed	6.38	19.47	26.24	101.28	BDL
P3	BC #1 Pond weed Wash	18.29	39.64	46.94	184.16	BDL
P4	BC #1 Before S. Curtain	21.49	35.76	26.09	94.83	5.63
P5	BC #1 Leaf Debris	7.50	20.74	11.39	55.60	BDL
P6	BC #1 Leaf Debris Wash	15.09	48.88	49.05	132.84	BDL
P7	BC #1 BSC Floating cattail Debris	32.33	68.39	33.02	110.89	2.22
P8	BC #1 BSC Floating cattail Debris Wash	27.77	41.22	17.75	133.65	3.54
P9	BC# 2 Plant 1	1.73	6.98	3.41	16.06	BDL
P10	BC# 2 Plant 1 Wash	1.66	7.22	3.14	21.69	BDL
P11	BC# 2 Chara	1.83	13.03	3.24	17.61	BDL
P12	BC# 2 Chara Wash	2.32	10.17	3.87	20.36	BDL
P13	BC #2 Potpus	1.73	14.09	3.95	12.50	BDL
P14	BC #2 Potpus Wash	1.87	9.59	2.37	13.31	BDL
P15	BC #2 nearshor crustose algae	1.47	12.95	13.99	11.92	BDL
P16	BC #2 nearshor crustose algae Wash	1.55	9.76	2.62	11.11	BDL
P17	BC #2 potpus	1.89	12.69	1.83	12.78	BDL
P18	BC #2 potpus Wash	1.59	8.83	3.46	11.69	BDL
P19	BC #2 dead cattail stock	1.22	10.32	7.18	11.95	BDL
P20	BC #2 dead cattail stock Wash	1.34	9.28	1.47	16.50	BDL
P21	BC #2 dead cattail surface	1.27	7.81	2.59	7.15	BDL
P22	BC #2 dead cattail surface Wash	1.43	8.27	1.71	8.72	BDL
P23	BC #1 cattail- Root	50.51	11.86	27.66	65.77	3.38
P24	BC #1 cattail- Rhizo	6.88	3.51	10.77	33.04	BDL
P25	BC #1 cattail- leaf	1.19	2.35	8.91	22.85	BDL
P26	BC #2 cattail- Root	34.89	64.23	10.51	68.48	3.07
P27	BC #2 cattail- Rhizo	1.89	4.10	2.69	23.05	BDL
P28	BC #2 cattail- leaf	0.70	2.26	2.57	10.80	BDL
P29	Dupe 1-BC #1	17.84	30.43	22.17	79.03	4.72
P30	Dupe 2- BC# 2 Chara	1.45	10.51	2.38	12.54	BDL
P31	Dupe 3- BC #2 potpus	1.35	10.79	2.86	9.87	BDL
P32	Dupe 4- BC #2 cattail- leaf	0.72	2.41	2.67	12.36	BDL
P33	Dupe 5- BC #2 cattail- Root	38.95	71.55	11.95	79.66	3.47
P34	BC #3 Algae	0.83	7.86	2.47	13.85	BDL
P35	BC #3 Algae Wash	1.27	6.26	5.19	20.53	BDL
P36	BC #3 Cara Inflow	3.94	18.44	3.31	33.76	BDL
P37	BC #3 Cara Inflow Wash	3.21	14.77	5.37	25.22	BDL
P38	BC #3 Aquatic moss	1.68	20.75	6.86	13.58	BDL
P39	BC #3 Aquatic moss Wash	1.67	12.28	2.42	15.20	BDL
P40	BC #3 Cara	2.66	19.88	4.78	40.75	BDL
P41	BC #3 Cara Wash	3.42	18.78	6.74	34.38	BDL
P42	BC #3 Stupee	2.89	14.76	9.14	51.73	BDL
P43	BC #3 Stupee Wash	6.10	15.72	39.52	70.82	BDL
P44	Cliff pond Potpus	0.77	8.10	2.12	15.00	BDL
P45	Cliff pond Potpus Wash	0.48	3.94	1.38	4.70	BDL
P46	Cliff pond grassy plant	1.60	13.44	26.51	44.83	BDL
P47	Cliff pond grassy plant Wash	BDL	BDL	BDL	79.52	BDL
P48	Cliff pond weed #2	1.48	12.88	2.91	38.58	BDL
P49	Cliff pond weed #2 Wash	1.67	10.73	3.33	34.29	BDL
P50	Cliff pond potnat	1.39	10.06	9.06	25.27	BDL
P51	Cliff pond potnat Wash	1.83	9.65	5.24	32.17	BDL
P52	Cliff pond algae bottom	2.47	10.97	2.56	23.95	BDL
P53	Cliff pond algae bottom Wash	3.66	14.68	5.05	43.80	BDL
P54	Cliff pond floating algae	7.98	20.03	9.28	54.98	2.36
P55	Cliff pond floating algae Wash	10.72	26.61	12.76	84.46	BDL
P56	Cliff pond tire algae	0.89	7.46	2.11	18.45	1.73

		mg/kg dry mass				
	Sample	Co	Ni	Cu	Zn	As
P57	Cliff pond tire algae Wash	3.39	18.08	8.06	63.98	21.43
P58	Cliff pond- Root	5.75	7.26	6.07	45.85	7.59
P59	Cliff pond- leaf	0.22	1.31	4.28	13.77	1.61
P60	Cliff pond plant - Root	10.07	34.50	23.06	282.09	12.13
P61	Cliff pond plant - leaf	0.26	1.64	3.89	23.92	BDL
P62	BC #1 Scripus- Root	12.61	36.17	45.12	88.67	2.56
P63	BC #1 Scripus- leaf	2.33	7.99	46.09	82.57	2.30
P64	Dupe 1-BC #3 Cara Inflow	3.73	17.26	3.32	31.70	1.96
P65	Dupe 2- BC #3 Stupee	3.43	17.35	11.51	61.46	2.04
P66	Dupe 3- Cliff pond potnat	1.58	11.47	12.14	29.10	BDL
P67	Dupe 4- Cliff pond algae bottom	2.65	11.71	2.53	26.04	BDL
P68	Dupe 5- Cliff pond- leaf	BDL	BDL	4.65	14.07	BDL
P69	SRM 1 - TORT 2	0.46	2.07	96.10	181.32	21.54
P70	SRM 2 -TORT 2	0.50	2.18	99.57	188.69	22.36
P71	SRM 1 - TORT 2	0.56	2.45	109.35	200.51	23.84
P72	SRM 2 -TORT 2	0.57	2.54	113.71	208.34	24.78
	Average	**6.27**	**16.23**	**16.25**	**53.85**	**8.16**
	s.d	**10**	**15**	**25**	**57**	**8**
	Certified Value	**0.51**	**2.50**	**106.00**	**180.00**	**21.60**
	MDL	**0.15**	**0.71**	**0.30**	**1.27**	**1.40**

Method- Microwave acid digestion ICP-MS

		mg/kg dry mass				
	Sample	Sr	Mo	Cd	Ba	Pb
P1	BC #1	38	BDL	BDL	128.09	19.94
P2	BC #1 Pond weed	175	BDL	BDL	19.84	1.67
P3	BC #1 Pond weed Wash	1014	BDL	BDL	123.08	22.11
P4	BC #1 Before S. Curtain	37	BDL	BDL	130.47	20.04
P5	BC #1 Leaf Debris	286	BDL	BDL	72.99	4.31
P6	BC #1 Leaf Debris Wash	183	BDL	BDL	98.71	24.83
P7	BC #1 BSC Floating cattail Debris	509	BDL	0.61	65.87	7.43
P8	BC #1 BSC Floating cattail Debris Wash	567	BDL	BDL	103.48	16.60
P9	BC# 2 Plant 1	3161	BDL	BDL	36.56	0.57
P10	BC# 2 Plant 1 Wash	4209	BDL	BDL	48.60	1.99
P11	BC# 2 Chara	3848	BDL	BDL	46.61	0.19
P12	BC# 2 Chara Wash	4107	BDL	BDL	49.82	2.81
P13	BC #2 Potpus	4443	BDL	BDL	45.59	0.48
P14	BC #2 Potpus Wash	3874	BDL	BDL	47.44	1.06
P15	BC #2 nearshor crustose algae	3990	BDL	BDL	40.34	0.50
P16	BC #2 nearshor crustose algae Wash	4093	BDL	BDL	41.85	1.44
P17	BC #2 potpus	4148	BDL	BDL	45.69	0.66
P18	BC #2 potpus Wash	3652	BDL	BDL	41.59	1.19
P19	BC #2 dead cattail stock	3441	BDL	BDL	32.90	1.05
P20	BC #2 dead cattail stock Wash	3903	BDL	BDL	37.75	1.96
P21	BC #2 dead cattail surface	3174	BDL	BDL	31.72	0.49
P22	BC #2 dead cattail surface Wash	4050	BDL	BDL	39.99	1.46
P23	BC #1 cattail- Root	60	BDL	BDL	18.74	13.79
P24	BC #1 cattail- Rhizo	40	0.66	BDL	12.89	3.32
P25	BC #1 cattail- leaf	47	BDL	BDL	8.94	0.76
P26	BC #2 cattail- Root	625	BDL	BDL	76.20	2.55
P27	BC #2 cattail- Rhizo	66	BDL	BDL	2.81	0.26
P28	BC #2 cattail- leaf	102	BDL	BDL	3.84	BDL
P29	Dupe 1-BC #1	34	BDL	BDL	116.35	16.85
P30	Dupe 2- BC# 2 Chara	2990	BDL	BDL	36.97	BDL

		mg/kg dry mass				
	Sample	Sr	Mo	Cd	Ba	Pb
P31	Dupe 3- BC #2 potpus	3448	BDL	BDL	35.11	0.35
P32	Dupe 4- BC #2 cattail- leaf	109	BDL	BDL	3.89	BDL
P33	Dupe 5- BC #2 cattail- Root	831	0.57	BDL	88.13	3.04
P34	BC #3 Algae	5291	BDL	BDL	49.33	0.47
P35	BC #3 Algae Wash	2679	BDL	BDL	34.34	1.82
P36	BC #3 Cara Inflow	3330	BDL	BDL	48.74	1.95
P37	BC #3 Cara Inflow Wash	4839	BDL	BDL	52.55	2.12
P38	BC #3 Aquatic moss	4497	BDL	BDL	41.84	0.73
P39	BC #3 Aquatic moss Wash	5333	BDL	BDL	51.85	1.94
P40	BC #3 Cara	1783	BDL	BDL	43.23	2.06
P41	BC #3 Cara Wash	1823	BDL	BDL	42.61	3.03
P42	BC #3 Stupee	858	BDL	BDL	26.09	1.75
P43	BC #3 Stupee Wash	1457	BDL	BDL	55.17	6.61
P44	Cliff pond Potpus	650	BDL	BDL	69.72	0.18
P45	Cliff pond Potpus Wash	534	BDL	BDL	57.67	0.25
P46	Cliff pond grassy plant	549	BDL	BDL	76.42	0.92
P47	Cliff pond grassy plant Wash	683	BDL	BDL	96.12	BDL
P48	Cliff pond weed #2	509	BDL	BDL	73.92	0.74
P49	Cliff pond weed #2 Wash	651	BDL	BDL	93.23	1.83
P50	Cliff pond potnat	495	BDL	BDL	76.99	0.81
P51	Cliff pond potnat Wash	508	BDL	BDL	93.85	2.49
P52	Cliff pond algae bottom	434	BDL	BDL	67.10	2.06
P53	Cliff pond algae bottom Wash	377	BDL	BDL	69.12	3.61
P54	Cliff pond floating algae	242	0.88	BDL	74.08	6.54
P55	Cliff pond floating algae Wash	155	BDL	BDL	84.97	12.72
P56	Cliff pond tire algae	518	0.57	0.69	63.45	0.59
P57	Cliff pond tire algae Wash	358	6.88	9.29	60.64	6.44
P58	Cliff pond- Root	26	2.33	2.78	10.30	5.00
P59	Cliff pond- leaf	4	0.90	0.69	1.29	0.23
P60	Cliff pond plant - Root	40	1.45	3.15	43.97	3.02
P61	Cliff pond plant - leaf	27	0.54	BDL	4.61	0.22
P62	BC #1 Scripus- Root	51	1.04	1.47	16.71	2.79
P63	BC #1 Scripus- leaf	129	1.38	1.22	12.49	0.93
P64	Dupe 1-BC #3 Cara Inflow	2999	0.54	0.67	41.84	1.87
P65	Dupe 2- BC #3 Stupee	984	0.62	0.84	24.80	1.95
P66	Dupe 3- Cliff pond potnat	572	BDL	BDL	86.72	0.89
P67	Dupe 4- Cliff pond algae bottom	464	BDL	BDL	69.93	2.05
P68	Dupe 5- Cliff pond- leaf	3	BDL	BDL	BDL	BDL
P69	SRM 1 - TORT 2	43	0.93	26.12	1.53	0.38
P70	SRM 2 -TORT 2	44	0.99	27.04	1.65	0.38
P71	SRM 1 - TORT 2	47	1.14	29.06	1.93	0.44
P72	SRM 2 -TORT 2	49	1.18	29.66	1.94	0.41
	Average	**1518**	**1.33**	**9.52**	**50.08**	**3.82**
	s.d	**1722**	**1**	**12**	**33**	**6**
	Certified Value	**45.20**	**0.95**	**26.70**		**0.35**
	g	**0.89**	**0.49**	**0.61**	**0.70**	**0.17**

Method- Microwave acid digestion ICP-MS BDL: Below Detection Limit

7. Sulphur concentrations in water and biomass

Water samples

Total Sulfur	mg/L
BC1 outflow	3250
BC2	3510
BC2 inflow	6990
BC2 outflow	5940
BC3	6060
BC3 inflow	7150
cliff pond 28	3430
cliff pond outflow	6020
H2O BC1 ASC	2690
H2O BC1 BSC	3110
inflow cliffs	4090

Method- ICP-AES

Chara samples

	% Sulfur
1-BC#2 Chara	0.37
2-BC#2 Chara wash	0.28
3-BC#3 Cara inflow	0.53
4-BC#3 Cara inflow wash	0.82
5-BC#3 Cara	0.59
6-BC#3 Cara wash	0.68

Method- Leco combustion elemental analyzer